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SURVIVAL AND DEVELOPMENT AT LOW TEMPERATURES OF EGGS AND PREINFECTIVE LARVAE OF HORSE STRONGYLES[1]

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INTRODUCTION

The preparasitic, or free-living phase, of the life cycle of the strongylid nematodes parasitic in horses includes the egg, the first- and second-stage preinfective larvae, and the ensheathed or infective larva. Among strongyles generally, these stages are vulnerable to the effects of environmental factors. Temperature, moisture, and sunlight are generally considered to be the most important natural influences affecting the survival and development of nematode eggs and larvae.

Studies in which the effects of but a single factor of the environmental complex are investigated either in laboratory or outdoor experiments have been criticized as academic and of doubtful practical significance, since in nature the various elements of the complex ordinarily function simultaneously as determinants of the survival and development of eggs and larvae. Field studies in which the effect of the environment as a whole may be determined are undoubtedly of great value. However, the results of such studies have validity under identical or very similar conditions only. Even in the same locality meteorological conditions rarely or never are duplicated from year to year. Moreover, by themselves, such studies cannot demonstrate which factor or factors in the complex caused the observed result or the degree to which each participated in the result. Hence, both types of investigation apparently have their limitations, and the formulation of generalizations, interpretations, and expressions of probability, so far as that is possible and advisable, should be based on the information elicited by investigations of both types.

In all experiments reported in this paper, desiccation, excess moisture, and direct sunlight were eliminated or minimized as causative factors and, consequently, temperature was the principal element in the causation of the observed experimental results.

The specific objectives of the investigation were to determine (1) the resistance of the eggs and preinfective larvae of horse strongyles to subfreezing temperatures; (2) the survival time of these stages at a mean temperature of 36° F., which presumably would inhibit appreciable development; and (3) the survival and development of these stages in feces exposed to temperatures prevailing during winter and early spring in the vicinity of the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., where all the experiments were conducted in 1938 and 1939.

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The resistance of infective horse strongyle larvae to cold was not studied since, as a result of work by other investigators, it appears to be well established that these larvae can endure very low winter temperatures. The observations on infective larvae that are included in this paper are, therefore, merely incidental to the principal objectives. The effects of cold on the eggs and preinfective larvae also have been investigated by others, but definite information on this subject appears to be lacking.

REVIEW OF LITERATURE

Albrecht (*1*)[2] stated that cold delays the development of horse strongyle eggs but does not exert an injurious effect on them. Hueber (*7*) reported that the eggs withstand cold for a long time. De Blieck and Baudet (*4*) found that cold hinders development of the eggs. They observed that eggs kept for 12 days at 6° C. did not hatch, but many contained living larvae. According to these authors, eggs in the morula stage kept at 0° C. for 12 days did not undergo development but retained their vitality. However, when exposed to temperatures ranging from −15° to −20° for 6 hours, all eggs were killed. Gackstatter (*6*) reported that hard freezing for 15 days of eggs in fecal balls, or for 10 days in feces that had been broken up, did not reduce the vitality of the eggs but merely arrested their development. This author also noted that eggs kept for 10 days at 2° to 3° C. were still capable of development.

According to Enigk (*5*), uterine eggs on agar cultures were not all killed at −8° C. in 45 days, but none developed after 56 days at this temperature. Enigk reported, however, that fresh fecal pellets kept at −8° for 55 days yielded several colonies of larvae when the feces were cultured. A few small colonies also developed in cultures consisting of pellets that had been broken up and exposed at this temperature for 41 days. Wetzel (*13*) stated that a rather high percentage of eggs of *Strongylus equinus* tolerated freezing temperatures near 0° C. for several hours.

Parnell (*9*) stated that horse strongyle eggs, before embryonation, are very resistant to the extremes of temperature and climate encountered in Canada. He reported that the continual low temperatures of a severe Canadian winter were insufficient to kill "sclerostome" eggs, that these temperatures merely inhibited development, and that the eggs embryonated and hatched normally in the spring. He also stated that embryonated eggs are much less resistant and are relatively easily killed. He gave no experimental basis for these statements but later (*10*) described an experiment in which feces containing eggs and feces containing other free-living stages were exposed to winter temperatures on Montreal Island for 3½ months, apparently from December 3, 1934, to April 1, 1935. The average mean temperature in that vicinity is given as −15° C. in January; daily fluctuations in temperature may be considerable. Cultures prepared in the spring from the feces that had originally contained unembryonated eggs yielded infective larvae. The experiment was interpreted in part as indicating the great resistance of the eggs to cold.

Baker, Salisbury, and Britton (*2*) reported recently that in an experiment in the vicinity of Ithaca, N. Y., overwintering of horse strongyle

[2] Italic numbers in parentheses refer to Literature Cited, p. 217.

eggs on pasture was apparently lethal to 68 percent of these eggs in manure deposited normally and to 100 percent of the eggs in feces spread in a thin layer.

Observations on the survival of preinfective horse strongyle larvae at low temperatures are scant. De Blieck and Baudet (*4*) found that all newly hatched rhabditiform larvae placed in water and exposed at −15° to −20° C. were killed in 6 hours. Wetzel (*13*) stated that a rather high percentage of preinfective larvae of *Strongylus equinus* tolerated freezing temperatures near 0° C. for several hours. Parnell (*9*) stated that the free-feeding larval stages are much less resistant than the eggs and are relatively easily killed. Later, on the basis of the experiment already mentioned, he (*10*) repeated this statement.

Parnell (*9*) also stated that frequent alternate freezing and thawing can be relied on to kill the free-living stages of horse strongyles. His previously mentioned experiment (*10*) was interpreted as indicating the susceptibility of all free-living stages of these parasites to violent and frequent fluctuations in temperature. Similar but somewhat more specific views were expressed by Britton (*3*) in a summary of the work of several investigators on the factors affecting the viability and development of free-living stages of horse strongyles. He stated that "alternate freezing and thawing can be relied upon to kill all preparasitic stages providing the thaw does not last longer than two weeks," and "during a thaw many eggs which are lying dormant on the field will hatch and the preinfective stages develop slowly and if the thaw is followed by freezing temperatures these forms will be killed as they are susceptible to these temperatures." Examination of the papers enumerated by Britton in connection with his summary has failed to reveal the exact counterpart of these statements. They represent in part his interpretation of the literature that he reviewed.

Wetzel (*13*) stated that an analysis of temperature data for a 30-year period showed that at Hanover, Germany, development of infective larvae of *Strongylus equinus* in the open seems possible from about the middle of March until the middle of November in a normal year.

To summarize, it has been shown in laboratory experiments that horse strongyle eggs are moderately resistant to subfreezing temperatures, but the degree of their resistance has not been defined precisely. The results of one of the two reported outdoor experiments on the effects of cold on the eggs appear to be inconsistent with the laboratory findings. Precise information as to the ability of the preinfective larvae to withstand freezing is lacking. It is known that these larvae are less resistant than the eggs, but the available evidence does not permit a more exact comparison. The ability of eggs and preinfective larvae to survive at temperatures slightly above the freezing point scarcely has been explored. The literature contains statements as to the susceptibility of all free-living stages to alternate freezing and thawing and to violent fluctuations in winter temperatures, but, in the writer's opinion, these statements rest on inference rather than on conclusive experimental evidence so far as the effects of these changes on eggs and preinfective larvae are concerned.

METHODS AND PROCEDURE

The feces used in this investigation, which included both laboratory and outdoor experiments, contained the free-living stages of species

of *Strongylus* and of the subfamily Cyathostominae, the latter predominating. It was not determined whether the free-living stages of these two closely related systematic groups react differently to low temperatures. The experimental results apply to the free-living stages of horse strongyles in general.

The feces used in all experiments were collected 2 hours or less after being passed. Feces collected from several horses were used in the investigation. However, only feces from the same collection and from one horse were used for the cultures of an individual experiment. In some cases feces of a single collection from one horse were used in more than one experiment. Immediately after collection, the fecal pellets were broken up, and weighed portions of the well-mixed fecal mass were placed in wide-mouthed glass bottles or jars.

In all experiments involving the exposure of eggs, the bottles containing the feces to serve as control cultures were immediately covered with glass lids, and after they had been kept for a week or more at room temperature (65°–85° F.), the feces were examined for infective larvae.

In the laboratory experiments the following procedure was used: To test the resistance of unembryonated eggs to subfreezing temperatures, the test bottles were corked, as a rule, and immediately placed in the freezing compartment of an electric household refrigerator. Exceptionally, glass lids were used to cover bottles during exposure. Following the desired exposure, the bottles were removed from the freezer and kept at room temperature for a week or more before the feces were examined for infective larvae. If the bottles had been corked during exposure, glass lids were substituted for the corks when the bottles were removed from the freezer.

To test the effect of embryonation on the resistance of the eggs to subfreezing temperatures, the procedure was essentially the same as in the experiments with the unembryonated eggs. For purposes of comparison, bottles of one series were immediately corked and placed in the freezer and bottles of a second series were covered with glass lids and kept at room temperature long enough to permit the eggs to embryonate. They were then corked and placed in the freezer.

To test the effect, on the eggs, of continuous exposure to a mean temperature of about 36° F., the test bottles were covered with glass lids and placed in a large cooler, the temperature of which was regulated with a thermostat. From time to time, as was necessary, water was added to each bottle to keep the feces moist. When, after exposure, the bottles were removed from the cooler they were kept at room temperature for at least a week before the feces were examined for infective larvae.

The procedures involved in determining the effects of repeated freezing and thawing were somewhat complicated and consequently are best shown in connection with the results. However, as in the procedures already described, the test bottles were corked while in the freezer but were covered with glass lids when they were removed for exposure to room temperature.

In experiments in which feces containing unembryonated eggs were placed outdoors to observe the effects of winter temperatures on the free-living stages, the test bottles were covered with glass lids at all times. The number of eggs per gram of the fresh mixed feces used for the preparation of cultures was determined. At intervals two or

three of the test bottles were brought indoors after identical exposure. When the bottles were brought into the laboratory, usually the egg count per gram of exposed feces, and invariably the number of larvae per culture, were determined. One bottle was kept at room temperature at least 1 week, and the feces were then examined for infective larvae.

In both the laboratory and outdoor experiments to test the effects of cold on preinfective larvae, the bottles containing the feces were covered with glass lids and kept at room temperature long enough to permit the desired stage to develop. The test cultures were then exposed, and the control cultures were immediately examined for larvae unless otherwise indicated in the tables. For exposure of preinfective larvae to subfreezing temperatures or to a mean temperature of about 36° F., other procedure was the same as in the respective experiments with eggs, except that the test cultures were examined only for surviving larvae immediately on removal from the freezer or cooler, unless otherwise indicated in the tables. At various intervals during the outdoor experiment, two identically exposed cultures usually were brought into the laboratory at a time. One was examined immediately for larvae and the other for infective larvae after it had been kept at room temperature for about a week.

To determine the temperatures to which feces were exposed in the freezing experiments, a maximum and minimum recording thermometer was kept in the compartment, as a rule. The temperature range was noted whenever cultures were removed from the freezer and the indices were reset. In some experiments, laboratory thermometers were kept in the compartment and the temperature was noted at frequent intervals. These thermometers and the metal back of the maximum and minimum thermometer were in contact with the metal coil in which the refrigerant circulated or with a thin layer of ice covering the coil. The bottoms and sometimes the sides of the bottles containing the feces were in contact with the same surface. The actual temperature of the feces was judged to be similar to that registered by the thermometers. It was noted, however, that the temperature of the air in the compartment was somewhat above that recorded by thermometers placed as has been described. A thermometer connected with the thermostat was frequently read to determine the variation in the temperature of the cooler.

A maximum and minimum thermometer was kept at the site of each outdoor experiment. The temperature range was noted each time that cultures were brought into the laboratory and the indices were reset.

The bottles involved in outdoor experiments, as well as the thermometer used to record temperatures, were in the shade at all times. Thus the feces were protected from direct sunlight. Since the bottles were covered, the feces were also protected from wetting by snow or rain and from appreciable desiccation. The possible inimical influences, therefore, so far as survival and development of the eggs and larvae are concerned, were largely limited to low temperatures and natural fluctuations in temperature.

The Baermann isolation apparatus was used throughout this investigation for the recovery of preinfective and infective larvae from feces. As noted elsewhere, in most experiments involving recovery of preinfective larvae, a piece of silk bolting cloth was placed on the

screen to reduce the passive descent of fecal particles and dead larvae. Fluid was not withdrawn from the hose for at least 48 hours after the feces had been placed on the screen. Usually the period that elapsed was much longer. Descent of larvae into the stem of the funnel of the Baermann apparatus was the criterion of their viability. Most of the preinfective larvae were dead when the fluid was examined as these larvae usually live only a short time in water, even under favorable conditions. Successive withdrawals of fluid from the rubber hose attached to the stem of the funnel were continued until samples were found to be negative for larvae when inspected under the binocular microscope. Following a process of sedimentation and centrifugation, the larvae recovered were concentrated in a small volume of water and counted. The counts were direct or by means of a dilution method, depending on the abundance of the larvae. A dilution technique was used in making egg counts.

In certain experiments it was necessary to determine the developmental stages of the larvae recovered from cultures or to count preinfective and infective larvae differentially. As already indicated, most preinfective larvae died after remaining in contact with the fluid in the Baermann apparatus for 48 hours. Third-stage larvae, on the other hand, survived very much longer. Therefore, in general all larvae that were dead or partly disintegrated at the time of examination were counted as preinfective and all that were alive and of normal appearance were counted as third stage. However, the size, general appearance, and structure of the larvae were also used in differentiating them. These criteria were likewise used in distinguishing the various stages of development among preinfective larvae—the first stage, second stage, or second ecdysis. The appearance and organization of the intestine were used to differentiate partly developed from fully developed third-stage larvae, but if there was any doubt as to whether a larva should be considered infective or preinfective, it was classified as infective.

In the examination of some cultures, after the usual differential count with the dissecting microscope had been completed, 0.075 cc. of a suspension of the larvae was also examined under a compound microscope and a second differential count of the larvae was made. Sometimes the larvae were treated with an iodine solution to aid in determining their morphological status. Usually the total number of larvae recovered from the culture was then calculated from the results of the first count under the dissecting microscope and the numbers or percentages of larvae in the various developmental stages were calculated from the differential counts obtained by using the compound microscope. The detailed results of these examinations are too voluminous for inclusion with the experimental data. Unless otherwise indicated in the text or tables, all figures given in connection with experiments on preinfective larvae are based on differential counts obtained with the dissecting microscope only.

LABORATORY EXPERIMENTS WITH EGGS

EFFECT ON SURVIVAL OF UNEMBRYONATED EGGS OF CONTINUOUS EXPOSURE TO SUBFREEZING TEMPERATURES AND TO A MEAN TEMPERATURE OF 36° F.

As shown in table 1, in experiment 1 a small percentage of unembryonated eggs in a culture continuously frozen for 49 days were still alive, but in experiment 2 all eggs in cultures frozen for 48 days or

longer were dead. A small percentage of eggs in feces frozen for 82 days were still alive in experiment 3.

TABLE 1.—*Survival of unembryonated horse strongyle eggs in feces continuously exposed, for indicated periods, to subfreezing temperatures and to a mean temperature of about 36° F.*

Experiment No.	Culture No.	Weight of feces	Period of exposure	Approximate range of temperatures at which cultures were exposed	Estimated mean temperature of entire exposure period	Infective larvae recovered	Apparent survival [1]
		Grams	*Days*	°F.	°F.	*Number*	*Percent*
1	1	75	7	14–30	21	17,700	92.9
	2		14	14–30		3,070	16.1
	3		21	14–30		7,150	37.5
	4		42	14–30		5,750	30.2
	5		49 [2]	14–30		1,110	5.8
	6		7	34–37	36	3,660	19.2
	7		14	34–37		12,520	65.7
	8		21	34–37		9,850	51.7
	9		62	34–37		8,000	42.0
	10		140	34–37		1,050	5.5
	11		[3]			28,300	
	12		[3]			9,800	
2	1	35	48	14–23	17	0	0
	2		54	14–23		0	0
	3		61	14–23		0	0
	4		68	14–23		0	0
	5		[3]			61,000	
	6		[3]			52,200	
	7		[3]			67,000	
	8		[3]			51,600	
3	1	35	14	10–30	14	2,500	8.7
	2		24	10–30		2,050	7.2
	3		31	8–30		440	1.5
	4		40	7–30		860	3.0
	5		56	6–30		735	2.6
	6		68	6–30		250	.9
	7		82	6–30		500	1.7
	8		24	34–37	36	1,675	5.8
	9		45	[4] 34–44		750	2.6
	10		68	[4] 34–44		3,850	13.4
	11		96	[5] 34–50		1,000	3.5
	12		114	[5] 34–50		1,200	4.2
	13		153	[6] 34–50		225	.8
	14		195	[6] 34–50		135	.5
	15		[3]			25,200	
	16		[3]			31,200	
	17		[3]			29,500	
4	1	30	29	34–37	36	4,400	46.7
	2		84	[7] 34–46		400	4.2
	3		99	[7] 34–46		250	2.7
	4		126	[7] 34–46		185	2.0
	5		155	[7] 34–46		38	.4
	6		169	[7] 34–46		34	.4
	7		[3]			17,000	
	8		[3]			1,280	
	9		[3]			13,250	
	10		[3]			9,400	
	11		[3]			2,000	
	12		[3]			13,600	

[1] Based on the average number of infective larvae recovered from the control cultures.
[2] Actually 50 days, but the culture had thawed beginning about 24 hours earlier owing to an interruption in electrical service.
[3] Control; unexposed.
[4] Maximum attained in the course of an 18-hour period beginning on the 28th day of exposure.
[5] Maximum attained in the course of a 5-day period beginning on the 69th day, but 43° F. was exceeded during only a brief part of this period; also subjected to maximum as shown in footnote 4 for cultures 9 and 10.
[6] Subjected to maximum of 42° F. on the 123d day of exposure and of 46° on the 129th day, each being recorded in the course of intervals of not more than 18 hours; also subjected to maxima as shown in footnote 5 for cultures 11 and 12.
[7] Maximum attained in the course of an 18-hour period beginning on the 69th day of exposure.

Cultures containing unembryonated eggs were also subjected to continuous freezing in connection with the experiments reported in tables 2 and 4. A small percentage of eggs in a culture frozen for 97

days yeilded infective larvae, as shown in table 2. In the experiment reported in table 4, about 28 days was the maximum exposure and, as in all other experiments, an exposure of this duration was not lethal to all eggs.

The proportion of eggs surviving roughly comparable periods of exposure in these five experiments varied widely, as is indicated by the relationships between the number of infective larvae recovered from exposed and control cultures. One factor contributing to this result probably was variation in the total number of eggs in the feces comprising the cultures of each experiment. This is regarded as a principal cause of the wide variation in the number of infective larvae recovered from the various control cultures of some of the experiments. Difference in extreme and mean temperatures of exposure probably was an additional factor in determining the proportion of eggs surviving in comparably exposed test cultures.

The experiments were, therefore, of limited quantitative value. They definitely showed, however, that continuous exposure to subfreezing temperatures ultimately exerted a pronounced lethal effect on unembryonated eggs. That only a small proportion of eggs, or none at all, survived exposures of about 7 to 8 weeks at a mean temperature of about 14° to 21° F., is also a warranted conclusion. That at a given range of subfreezing temperature the number of surviving eggs decreased gradually as the duration of exposure increased, is strongly suggested by the data of these experiments as a whole.

In the experiments involving exposure of unembryonated eggs in feces to a mean temperature of about 36° F., very large proportions were killed by prolonged exposure, as shown in table 1. A small proportion survived an exposure of 195 days in one experiment. In the remaining two experiments the maximum periods of exposure were 140 and 169 days, and these exposures also did not kill all the eggs. In two of the three experiments, about one-half of the eggs were still alive after being kept at about 36° for 3 to 4 weeks. In general, the data strongly suggest that decrease in the number of viable eggs and increase in duration of exposure were related. But because of variability in results, the experiments do not warrant quantitative conclusions with respect to the rate of devitalization of the eggs.

EFFECT OF EMBRYONATION ON RESISTANCE OF EGGS TO SUBFREEZING TEMPERATURES

Embryonated eggs were much more readily killed than unembryonated eggs in feces exposed to the same range of subfreezing temperatures (7° to 32° F.), as shown in table 2. That cultures 7 to 12, after incubation in the laboratory for 19 hours, contained mainly embryonated eggs when exposed was shown by examination of an identically incubated culture of the same lot. Four grams of feces was removed from this culture for an egg count. From the remaining 26 gm. only about 500 preinfective larvae were recovered. The egg count showed that the feces contained about 900 eggs per gram, that a majority of the eggs contained larvae, and that many of the remaining eggs were in the tadpole stage of development. Since there was a small residue of segmenting eggs in the feces, these unembryonated eggs probably were the source of many of the infective larvae recovered from cultures 7 to 12 after the latter had been exposed and incubated at room

temperature. The experiment does not permit a conclusion as to the absolute resistance of the embryonated eggs.

TABLE 2.—*Comparison of the survival of unembryonated and embryonated eggs in 30-gm. fecal cultures continuously exposed, for indicated intervals, to subfreezing temperatures*

Culture No.	Period of exposure	Approximate range of temperatures at which cultures were exposed [1]	Infective larvae recovered	Apparent survival [2]
	Days	*°F.*	*Number*	*Percent*
CULTURES CONTAINING ONLY UNEMBRYONATED EGGS (16- TO 64-CELL STAGES)				
1	13	9–26	2,950	31.3
2	29	7–26	725	7.7
3	34	7–26	2,370	25.2
4	47	7–32	850	9.0
5	56	7–32	230	2.4
6	97	7–32	34	.4
CULTURES CONTAINING MAINLY EMBRYONATED EGGS [3]				
7	11	9–26	1,000	10.6
8	28	7–26	170	1.8
9	33	7–26	50	.5
10	46	7–32	80	.8
11	55	7–32	38	.4
12	96	7–32	1	.01

[1] Estimated mean temperature of entire period of exposure, 16° F.
[2] Based on the average number of infective larvae recovered from the control cultures. The control cultures for this experiment were the same as for experiment 4 in table 1.
[3] See text.

EFFECT OF ALTERNATE FREEZING AND THAWING OF FECES

Survival of Unembryonated Eggs at Subfreezing Temperatures

To determine whether a repetition of the physical changes involved in freezing and thawing of feces would affect the resistance of unembryonated eggs to subfreezing temperatures, the various test cultures were frozen, removed from the freezer, thawed at room temperature, and frozen again from 3 to 10 times before they were finally incubated in the laboratory. To prevent appreciable development of the eggs during the periods of thawings, the cultures were allowed to remain at room temperature only until all ice crystals had melted and the feces had attained their normal consistency.

Resistance of the eggs to a mean temperature of about 14° F. was not reduced by repeated freezing and thawing of the feces. This fact is indicated by comparison of the number of infective larvae recovered from test cultures (table 3) and from those prepared from the same lot of feces but continuously frozen at similar temperatures (table 2). The mean subfreezing temperature of exposure of the series that was alternately frozen and thawed was lower than that of the continuously frozen series.

It was impracticable to determine the actual temperature of the feces during the various thawings. However, it is evident from the results of the experiment that the procedure employed prevented appreciable development of the eggs during these periods, for embryonation decreases the resistance of the eggs to subfreezing temperatures, as has been shown previously.

TABLE 3.—*Survival of unembryonated eggs at subfreezing temperatures as affected by repeated freezing and thawing of 30-gm. cultures of feces*

Culture No.	Total duration of exposure to subfreezing temperatures	Approximate range of temperatures at which cultures were exposed[1]	Times cultures were—		Intervals between (1) initial exposure to freezing and (2) thawings	Total time cultures were at room temperature for thawing	Period cultures were frozen after last thawing	Infective larvae recovered after incubation following final freezing	Apparent survival[2]
			Thawed	Frozen					
	Days	°F.	*Number*	*Number*	*Days*	*Hours*	*Days*	*Number*	*Percent*
1	12.75	14–21	3	4	0.75; 6; 11.5	6.0	1.25	4,300	45.6
2	28.50	14–21	4	5	0.75; 6; 11.5; 14.75	7.5	13.75	3,000	31.8
3	33.50	10–21	5	6	0.75; 6; 11.5; 14.75; 29.	9.0	4.50	1,600	17.0
4	46.50	10–21	6	7	0.75; 6; 11.5; 14.75; 29; 34.	10.5	12.50	650	6.9
5	55.50	7–21	7	8	0.75; 6; 11.5; 14.75; 29; 34; 47.	12.0	8.50	875	9.3
6	96.25	7–21	10	11	0.75; 6; 11.5; 14.75; 29; 34; 47; 56; 61.5; 83.75.	17.5	12.50	100	1.1

[1] Estimated mean temperature of entire exposure period, 14° F.
[2] Based on the average number of infective larvae recovered from the control cultures; the control cultures for this experiment were the same as for experiment 4 in table 1.

POTENTIAL YIELD OF INFECTIVE LARVAE

As previously reported, embryonated eggs were found to be more susceptible to subfreezing temperatures than unembryonated eggs. In experiments described later, nearly all preinfective larvae were killed by subfreezing temperatures in about 4 days and many were killed by much briefer exposures. These facts suggest that horse feces passed outdoors in regions where freezing and thawing alternate frequently in winter and early spring are largely "sterilized" during these seasons. It would seem that development would occur during periods of moderate temperatures and that succeeding subfreezing temperatures would readily kill the more susceptible stages mentioned. However, the degree of development and destruction would be determined by the relationships prevailing between the variables of temperature and time.

To obtain more information on this point, an experiment was performed in which feces originally containing unembryonated eggs were alternately frozen and thawed under laboratory conditions. This experiment differed from the freezing and thawing experiment already described in that the feces not only were thawed at room temperature but also were kept in the laboratory long enough to permit some development of the free-living stages each time that the cultures were removed from the freezer. The objective was to obtain the greatest possible lethal effect. Although outdoor fluctuations in temperature were simulated in the experiment, the transitions from subfreezing temperature to temperatures favorable for development were more rapid than those that occur in nature.

The yield of infective larvae from cultures subjected to repeated alternate freezing and thawing under the conditions of this experiment was reduced to a very small percentage of their potential yield, as shown in table 4, series C. In fact, culture 1 of series C, which was frozen for about 5 days, transferred to room temperature for 2 days, and again frozen for 5 days yielded, on incubation, a negligible number

TABLE 4.—*Comparison of the effects of alternate freezing and thawing and of continuous freezing on the yield of infective larvae from 30-gm. cultures initially containing unembryonated eggs*

SERIES A.—ALTERNATELY FROZEN AND THAWED AND EXAMINED IMMEDIATELY FOLLOWING FINAL PERIOD OF THAWING

Culture No.	Times culture was frozen	Duration of freezing period No.—					Total period of freezing	Range of freezing temperature	Times culture was thawed	Duration of thawing period No.—				Total period of thawing	Larvae recovered		Yield of infective larvae as compared with potential yield [1]
		1	2	3	4	5				1	2	3	4		Pre-infective	Infective	
	Number	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*°F.*	*Number*	*Days*	*Days*	*Days*	*Days*	*Days*	*Number*	*Number*	*Percent*
1	1	4.8					4.8	15–32	1	2.0				2.0	1,000	0	
2	2	4.8	4.9				9.7	15–32	2	2.0	1.1			3.1	81	57	
3	3	4.8	4.9	1.1			10.8	14–32	3	2.0	1.1	1.1		4.2	39	17	
4	4	4.8	4.9	1.1	1.8		12.6	7–32	4	2.0	1.1	1.1	3.0	7.2	4	19	

SERIES B.—ALTERNATELY FROZEN AND THAWED AND EXAMINED IMMEDIATELY FOLLOWING FINAL PERIOD OF FREEZING

1	2	4.8	4.9				9.7	15–32	1	2.0				2.0	7	0	
2	3	4.8	4.9	1.1			10.8	14–32	2	2.0	1.1			3.1	5	2	
3	4	4.8	4.9	1.1	1.8		12.6	10–32	3	2.0	1.1	1.1		4.2	4	1	
4	5	4.8	4.9	1.1	1.8	8.0	20.6	7–32	4	2.0	1.1	1.1	3.0	7.2	1	11	

SERIES C.—ALTERNATELY FROZEN AND THAWED AND EXAMINED FOLLOWING INCUBATION AFTER FINAL PERIOD OF FREEZING

1	2	4.8	4.9				9.7	15–32	1	2.0				2.0	0	92	1.0
2	3	4.8	4.9	1.1			10.8	14–32	2	2.0	1.1			3.1	0	50	.5
3	4	4.8	4.9	1.1	1.8		12.6	10–32	3	2.0	1.1	1.1		4.2	0	51	.5
4	5	4.8	4.9	1.1	1.8	8.0	20.6	7–32	4	2.0	1.1	1.1	3.0	7.2	0	33	.4

SERIES D.—FROZEN CONTINUOUSLY AND EXAMINED FOLLOWING INCUBATION AT ROOM TEMPERATURE

1	1	11.7					11.7	13–32	0					0	0	3,500	37.6
2	1	14.0					14.0	8–32	0					0	0	1,900	20.4
3	1	16.8					16.8	8–32	0					0	0	1,800	19.3
4	1	27.7					27.7	7–32	0					0	0	535	5.7

SERIES E.—UNEXPOSED CULTURES

1																8,500	
2																11,350	
3																10,500	
4																5,850	
5																10,600	
6																9,100	

[1] Based on the average number of infective larvae recovered from the control cultures.

of infective larvae. Evidently this culture contained principally embryonated eggs when frozen the second time, for culture 1 of series A, after also being frozen once and incubated in the laboratory for 2 days, yielded but a small number of preinfective larvae as compared with the average number of infective larvae recovered from the unexposed control cultures. Moreover, these preinfective larvae were nearly all killed by the second period of freezing, as shown by the results obtained for culture 1 of series B. A few infective larvae developed in the actual course of the alternate freezing and thawing treatment, as shown by the results obtained with cultures 2, 3, and 4, series A. Some of these infective larvae survived the various periods of freezing.

The recovery of comparatively large numbers of larvae from the continuously frozen cultures (series D) shows that the development, during the thawing periods, of stages more susceptible than the unembryonated egg was responsible for the far greater lethal effect observed in the cultures alternately frozen and thawed.

The incidental evidence of this experiment on the susceptibility to freezing of preinfective and newly developed infective larvae is in agreement with the results of later experiments. That this experiment also provides evidence on the resistance to freezing of unembryonated eggs has been previously pointed out.

OUTDOOR EXPERIMENTS WITH EGGS

Two experiments in which freshly passed feces were subjected to outdoor temperatures were carried on in the winter of 1938–39. The results of the first of these experiments are given in table 5. The egg counts on four of the control cultures of this experiment appeared to show a fairly even distribution of the eggs in the feces from which cultures were prepared. However, the number of infective larvae recovered from four other control cultures varied widely. Also, in the test cultures, there was no apparent relationship between the potential yield of infective larvae and the duration of exposure. Hence, the experiment failed to demonstrate quantitatively that the potential ability of the cultures to yield infective larvae was reduced by exposure to outdoor winter and spring temperatures.

Nevertheless, the following inferences from other data of the experiment are considered warranted: A few eggs in the feces became embryonated in the first 12 days of exposure, during which the maximum temperature recorded was 46° F., and many became embryonated and hatched in the first 22 days of exposure, during which 56° was the maximum temperature recorded. Embryonation was directly observed. That eggs hatched was indicated by a decrease of more than one-third in their number. Since a corresponding number of preinfective larvae was not found in culture 8, examined after 22 days of exposure, many larvae evidently were killed soon after hatching. Later in the course of the first 55 days of exposure, more eggs apparently hatched, but only a small number of larvae succeeded in reaching the infective stage. Hence, many more preinfective larvae apparently were killed during the last 33 days of this period. Thereafter, when temperatures more favorable for development were attained periodically, the number of viable eggs in the feces diminished further, and despite frequent alternations between subfreezing and moderate

temperatures and rather extensive and rapid fluctuations in temperature, preinfective and infective larvae increased in number. The developmental process had not been completed when the experiment was terminated on April 7, after the feces had been exposed 113 days. There was no evidence that unembryonated or embryonated eggs were killed in appreciable numbers under the conditions of this experiment.

TABLE 5.—*Survival and development of free-living stages of horse strongyles in 35-gm. cultures of feces containing unembryonated eggs when placed outdoors on December 15, 1938*

Culture No.	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Eggs in feces when removed to laboratory		Larvae recovered from culture when removed to laboratory			Infective larvae recovered after incubation of culture at room temperature for week or more
				Per gram	Condition	Preinfective	Infective	Total	
	1938	*Days*	°*F.*	*Number*[1]		*Number*	*Number*	*Number*	*Number*
1	Dec. 27	12	25–46	1,450	A few embryonated; remainder apparently viable.				
2						[2] 115	0	[2] 115	
3									1,900
4	*1939*			1,000	About one-third embryonated; remainder apparently viable.				
5	Jan. 2	17.7	14–56			[2] 40	0	[2] 40	
6									10,750
7				933	Majority embryonated; remainder apparently viable.				
8	Jan. 6	22	33–55			38	0	38	
9									3,750
10				915	do				[4] 10,000
11	Jan. 19	35	[3] 9–60			215	0	215	
12									10,500
13				465	Majority embryonated; viability of remainder questionable.	[4] 162	[4] 15	[4] 177	
14	Feb. 8	54.7	18–46						7,350
15				50	Apparently nonviable	[4] 1,150	[4] 162	[4] 1,312	
16	Feb. 28	75	20–70						1,925
17				50	do	[4] 26	[4] 544	[4] 570	
18	Apr. 7	113	22–78						1,700
19	[5]								2,900
20	[5]								1,300
21	[5]								22,300
22	[5]								3,000

[1] Egg counts on 4 control cultures were: 1,370; 1,580; 1,580; 1,700. All figures are the average of counts on two 4-gm. samples from each culture.
[2] Regarded as hatching from embryonated eggs while the feces were in the Baermann apparatus.
[3] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred).
[4] 8 gm. of feces had been removed from this culture for egg counts. The actual number of larvae recovered from the remainder of the culture was adjusted to the 35-gm. basis.
[5] Unexposed; control.

The most important facts shown by this experiment are as follows: Feces placed outdoors when fresh yielded appreciable numbers of infective larvae in the course of an exposure of about 3½ months to the fluctuating subfreezing and moderate temperatures prevailing in winter and early spring in the vicinity of Beltsville, Md., and were still a potential source of additional infective larvae early in April. From January 13 to 21, the cultures and the thermometer at the site of the experiment were partly or completely covered with

snow. During this time the actual temperatures of exposure undoubtedly did not correspond with air temperatures. At no other time during this experiment were more than traces of snow on the ground.

It was observed at the site of the outdoor experiments that air temperatures below and above the freezing point often occurred during the same day and that sometimes transitions from moderate subfreezing temperatures to those fairly favorable for development took place within 6 or 7 hours. The exact frequency of such temperature fluctuations was not recorded, however. Daily maximum and minimum temperatures recorded by an instrument located about 1 mile from the site of the experiments indicated that subfreezing temperatures occurred on 83 of the 132 days from December 15, 1938, to April 26, 1939, the period that the outdoor experiments were in progress. On 77 of these 83 days, thawing temperatures also were recorded. Fluctuations from about 20° or 25° F. to about 50° or 60° during December and January and from a little below freezing to about 60° or 70° in February and March occurred several times within periods of about 8 to 24 hours. Since the maximum and minimum temperatures recorded on the thermograph tended to differ somewhat from those observed during the same periods at the site of the experiments, the facts mentioned are not admissible as data of the experiments. However, they indicate that the experimental results were obtained in an area where frequent alternations of freezing and thawing temperatures and violent and rapid fluctuations in temperature were characteristic of the period of the experiments.

In the second experiment, begun 42 days later than the first, the outdoor exposure caused a marked reduction by spring in the number of infective larvae that developed in cultures (table 6) as compared with the potential yield (controls in table 4). Cultures exposed 90 days ending April 26, 1939, yielded infective larvae representing, on the average, slightly less than 10 percent of the average number recovered from the six control cultures. Many of the results obtained in the first experiment also are applicable to this experiment. An appreciable decrease in the number of eggs in the feces took place in the initial 13 days of exposure, during which the maximum temperature was only 46° F. That nearly all larvae hatching in this period were promptly killed is indicated not only by the failure to find more than a few survivors, but also by the fact that the percentage of reduction in the potential yield of infective larvae (37 percent) was only a little less than the observed decrease in the number of eggs (42 percent).

In the ensuing period of 20 days (from February 8 to 28), characterized by alternations between temperatures a few degrees below freezing and those favorable for moderately rapid development, the potential yield of infective larvae was still more markedly reduced. As in the previous experiment, however, some infective larvae developed in this period. This was apparently due partly to the fact that temperatures remained continuously above the freezing point for considerable periods and partly to the fact that although freezing at intervals killed some preinfective larvae, the temperatures were not low enough or sufficiently prolonged to kill all of them.

TABLE 6.—*Survival and development of free-living stages of horse strongyles in 30-gm. cultures of feces containing unembryonated eggs when placed outdoors on January 26, 1939* [1]

Culture No.	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Eggs in feces when removed to laboratory		Larvae recovered from culture when removed from laboratory [2]			Infective larvae recovered after incubation of culture in laboratory for week or more
				Per gram	Condition	Preinfective	Infective	Total	
	1939	*Days*	*°F.*	*Number* [3]		*Number*	*Number*	*Number*	*Number*
1	Feb. 8	12.7	22-46	483	Some embryonated	12	0	12	5,850
2									
3	Feb. 28	32.7	20-70	100	Apparently dead	370	7	377	685
4									
5	Mar. 17	49.7	23-70	100	do	68	16	84	850
6									
7	Mar. 24	57.0	22-78	100	do	200	4	204	1,065
8									
9	Apr. 7	71.0	29-78	100	do	10	295	305	960
10									
11	Apr. 26	90.0	[4] 30-88			0	1,050	1,050	
12						0	800	800	690
13									

[1] The control cultures for this experiment were the same as for the experiment reported in table 4.
[2] Except for cultures 11 and 12, the data were calculated from the number of larvae recovered from 22 gm. of feces after 8 gm. had been removed from the culture for egg counts.
[3] Egg counts on 4 control cultures were: 800; 900; 1,400; 1,600. All figures are the average of counts on two 4-gm. samples from each culture.
[4] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred).

The experiment provides no definite evidence of pronounced lethal action later in the exposure period, but the number of infective larvae in the cultures did not reach a maximum until the onset of fairly continuous moderate temperatures. The data indicate that some eggs were killed in this experiment. Although all the eggs in cultures 3, 5, 7, and 9 were considered to be dead, the number of infective larvae recovered from the comparably exposed cultures 4, 6, 8, and 10, respectively, exceeded the number of preinfective and infective larvae in the former group.

The cultures of the previous experiment were exposed in part to the same conditions that clearly resulted in a reduction in yield of infective larvae in the cultures of the present experiment. That a similar result was not definitely apparent in the former experiment was probably due to uneven distribution of the eggs in the feces comprising the cultures.

LABORATORY EXPERIMENTS WITH PREINFECTIVE LARVAE

SURVIVAL OF LARVAE IN EARLY STAGES OF DEVELOPMENT

Three experiments were performed in which fecal cultures were kept at room temperature for 42 to 53 hours before being exposed to subfreezing temperatures. All larvae recovered from the control cultures placed in the Baermann apparatus when the test cultures were exposed were either first stage or second stage. In experiment 1, first-stage larvae predominated. In experiments 2 and 3 the two stages were about equally represented.

Considerably more than 90 percent of these preinfective larvae were killed in 1.2 to 4 days at a mean temperature of 14°–21° F., as shown

in table 7. The results of experiment 1 are regarded as less accurate than those of experiments 2 and 3, since in examining the cultures of the latter two experiments a piece of silk bolting cloth was placed over the screen of the Baermann apparatus to minimize the descent of dead larvae. Larvae were recovered from cultures exposed much longer than 4 days, but this fact is attributed to the presence in the feces of a residue of viable eggs. Apparently some of these eggs hatched while the feces were in the Baermann apparatus. As shown in table 7, culture 1 of experiment 2 yielded only 150 surviving larvae, but when culture 2, which was exposed slightly longer, was incubated in the laboratory for a week it yielded more than 1,000 infective larvae. This finding indicates that the cultures of this experiment contained about 1,000 eggs that had not undergone appreciable development when the test cultures were exposed. It is well known that the development of the free-living stages of strongyles does not proceed at precisely the same rate in all parts of an ordinary culture, and some viable eggs still may be present in a culture that contains infective larvae.

TABLE 7.—*Survival of preinfective larvae, in early stages of development, in 70-gm. cultures of feces continuously exposed to subfreezing temperatures or at about 36° F.*

Experiment No.	Culture No.	Period of exposure	Approximate range of temperatures at which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered [1]	Apparent survival [2]
		Days	*° F.*	*° F.*	*Number*	*Percent*
1	1	4.0	14–30	19	1,500	5.3
	2	10.1	14–30		[3] 2,775	9.7
	3	17.9	14–30		1,280	4.5
	4	19.0	14–30		[4] 280	
	5	[5] 25.0	14–30		16	.06
	6	4.0	34–37	36	21,700	76.0
	7	17.9	34–37		26,400	92.5
	8	38.1	34–37		7,100	24.9
	9	80.1	34–37		1,900	6.7
	[6] 10	115.9	34–37		[7] 3	.01
	[6] 11	130.1	34–37		52	.2
	12	[8]			32,000	
	13	[8]			25,100	
2	1	3.8	14–32	21	150	.4
	2	4.0	14–32		[4] 1,050	
	3	6.0	14–32		10	.02
	4	11.9	14–32		26	.06
	5	19.0	14–32		15	.04
	6	33.8	14–32		7	.02
	7	47.8	14–32		2	.005
	8	58.1	14–32		0	0
	9	[8]			40,300	
	10	[8]			[4] 47,500	
3	1	1.2	7–18	14	8,775	4.4
	2	1.3	7–18		2,700	1.4
	3	4.1	7–18		29	.01
	4	5.0	7–18		67	.03
	5	13.1	7–18		122	.06
	6	13.1	7–18		55	.03
	7	[8]			194,000	
	8	[8]			205,000	

[1] Preinfective unless otherwise noted.
[2] Based on the average number of larvae recovered from the control cultures.
[3] Culture placed in Baermann apparatus 18 hours after removal from freezer.
[4] Infective larvae; culture incubated at room temperature for a week or more before examination.
[5] Actually 26 days, but electrical service was interrupted on the 25th day and culture had thawed when it was removed.
[6] Culture moistened on 80th day of exposure.
[7] Infective larvae developing during exposure.
[8] Unexposed; control.

Also in experiment 1, identically incubated cultures were exposed to a mean temperature of about 36° F. More than 90 percent of the

larvae in a culture exposed for 80 days to this temperature died, as shown in table 7. Probably the cultures remaining in the cooler at this time were moistened too much, for few larvae were recovered from them when they were examined thereafter. A large proportion of the larvae survived for 18 days at this temperature, and approximately one-fourth survived for 38 days. Exhaustion of the food granules of the intestinal cells was noted in larvae surviving prolonged exposure. A few larvae reached the infective stage in a culture kept for 116 days at about 36°. Similar results were obtained in the first series of cultures of the experiment reported in table 9, although in that experiment somewhat larger percentages of larvae survived rather comparable exposures and more infective larvae developed while the cultures were kept at about 36°. However, only a general similarity in the results of the two experiments could be expected because the cultures of the first series in table 9 initially contained some infective larvae and some larvae in the second ecdysis.

SURVIVAL OF LARVAE IN ADVANCED STAGES OF DEVELOPMENT

Very large proportions of larvae principally in advanced phases of the second stage or in the second ecdysis, but not yet infective, were killed by comparatively brief exposure to subfreezing temperatures, as shown in table 8. Infective larvae in the cultures also were not very resistant to such temperatures. It was not anticipated that appreciable development of the larvae would occur at a mean temperature of about 36° F. Hence in examining the control cultures the larvae were separated into only two groups. Those that were obviously still preinfective and those regarded as infective were differentially counted; larvae regarded as of doubtful infectivity were included in the latter group. Similarly, in the classification and counting of the larvae from exposed cultures, larvae of doubtful infectivity were included with those definitely infective. In examining cultures 8, 9, and 10, exposed for about 16 to 33 days to a mean temperature of 36°, it was noted that the number of larvae that were infective or doubtfully infective had markedly increased over those in the control cultures. In the cultures kept at a mean of 36° for 3 months or longer, not only had a large percentage of larvae survived but nearly all definitely had reached the infective stage. The number of infective larvae recovered from culture 11 practically equaled the average number of larvae of all stages recovered from the control cultures. Temperatures above 37° were attained during only a few hours of the exposure period.

Another experiment was performed to test these results. The survival and development of larvae in a series of cultures containing mainly first-stage and second-stage larvae and one containing mainly larvae undergoing the second ecdysis were compared. Cultures of the first series contained a small number of infective larvae when exposed, but about 18 percent of the larvae in cultures of the second series were infective when exposed, as indicated by examination of a control culture of each series. The number and proportion of infective larvae increased in cultures of both series while the feces were kept at a mean temperature of about 36° F., as shown in table 9. This result is in

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TABLE 8.—*Survival of preinfective larvae, in advanced stages of development, in 35-gm. cultures of feces incubated at room temperature for 74 hours before exposure to subfreezing temperatures or at about 36° F.*

Culture No.	Period of exposure	Approximate range of temperatures to which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered			Apparent survival [2]
				Preinfective [1]	Infective	Total	
	Days	*°F*	*°F.*	*Number*	*Number*	*Number*	*Percent*
1	1.74	14–20	16	830	[3] 170	1,000	15.5
2	2.66	14–20		150	50	200	3.1
3	3.74	14–20		400	65	465	7.2
4	15.73	14–20		0	4	4	.06
5	17.66	14–20		1	31	32	.5
6	17.66	14–20		0	26	26	.4
7	30.71	7–20		[4] 23	28	51	.8
8	15.73	34–37	36	1,030	[3] 5,720	7,350	113.7
9	22.66	34–37		115	[3] 1,010	1,125	17.4
10	32.92	34–37		350	[3] 3,150	3,500	54.1
11	88.75	[5] 34–42		225	6,225	6,500	100.5
12	88.75	[5] 34–42		500	3,790	4,290	66.3
13	142.87	[5] 34–42		0	2,680	2,680	41.4
14	([6])			5,550	[3] 650	6,200	
15	([6])			6,550	[3] 650	7,200	
16	([6])			5,500	[3] 500	6,000	

[1] Mainly in advanced phases of the second stage and in various phases of the second ecdysis, unless otherwise noted.
[2] Based on the average number of larvae of all stages recovered from the control cultures.
[3] Including some doubtfully infective larvae, but proportion undetermined.
[4] Living first-stage larvae regarded as having hatched from viable eggs while the feces were in the Baermann apparatus.
[5] During a period of about 12 hours on the 73d day of exposure, electrical service was interrupted and the temperature of the cooler rose for a few hours to the maximum shown.
[6] Unexposed; control.

TABLE 9.—*Comparison of survival of preinfective larvae in two series of 30-gm. cultures of feces incubated for different periods at room temperature before exposure to subfreezing temperatures and to about 36° F.*

INCUBATED IN LABORATORY FOR 50 HOURS AND CONTAINING MAINLY FIRST-STAGE AND SECOND-STAGE LARVAE WHEN EXPOSED

Culture No.	Period of exposure	Approximate range of temperatures to which culture was exposed	Estimated mean temperature of entire exposure period	Larvae recovered [1]			Apparent survival [2]	Relation between number of infective larvae recovered from test and control cultures
				Preinfective	Infective	Total		
	Days	*°F.*	*°F.*	*Number*	*Number*	*Number*	*Percent*	*Percent*
1	1.9	8–24	15	1,350	52	1,402	4.3	80.0
2	2.7	8–24		400	20	420	1.3	30.8
3	3.8	8–24		28	2	30	.1	3.1
4	14.9	34–37	36	30,900	100	31,000	95.1	153.8
5	43.7	[3] 33–50		16,135	815	16,950	52.0	1,253.8
6 [4]	93.7	[3] 33–50		980	120	1,100	3.4	184.6
7	([5])			32,535	65	32,600		

INCUBATED IN THE LABORATORY FOR 73 HOURS AND CONTAINING MAINLY LARVAE IN THE SECOND ECDYSIS WHEN EXPOSED

8	1.0	8–24	15	950	1,100	2,050	13.6	40.0
9	2.0	8–24		970	550	1,520	10.1	20.0
10	3.8	8–24		53	23	76	.5	.8
11	13.9	34–37	36	12,250	6,400	18,650	123.5	232.7
12	42.8	[3] 33–50		8,400	7,500	15,900	105.3	272.7
13 [4]	92.8	[3] 33–50		2,625	4,000	6,625	43.9	145.5
14	([5])			12,350	2,750	15,100		

[1] Total numbers of larvae recovered from cultures 5, 7, 12, 13, and 14 were calculated from the usual dilution counts by the use of the dissecting microscope, but the numbers of preinfective and infective larvae were calculated from differential counts obtained by the use of the compound microscope.
[2] Based on average number of larvae of all stages recovered from control culture.
[3] Early in the third week of exposure a maximum of 43° F. was reached during an 18-hour interval, and during a 5-day period in the 6th week of exposure a maximum of 50° was reached for a few hours, but during most of this period the temperature ranged from 34° to 37°.
[4] Moistened with water on the 67th day of exposure.
[5] Unexposed; control.

general agreement with those of previous experiments. Owing to unavoidable circumstances the maximum temperature was somewhat higher in the present experiment.

Observations not included in the table also indicated that about 1.4 percent of the larvae in cultures of the first series and about 50 percent of the larvae in cultures of the second series were in a late phase of the second ecdysis when exposed. During exposure to a mean temperature of about 36°, the percentage of larvae in this phase increased in cultures of the first series and decreased in those of the second. This finding is further evidence of development at that temperature. The proportions of larvae surviving comparable exposures at this temperature were considerably higher in the second series of cultures. Apparently this result was due to the greater ability of larvae in the second ecdysis to complete their development to the infective stage at a mean temperature of about 36°. The survival in cultures 6 and 13 was lower than was expected; apparently too much water had been added to these cultures on the sixty-seventh day of exposure. First-stage and second-stage larvae apparently were more susceptible to the effects of excess moisture than the more advanced stages. That nearly all preinfective larvae in all stages are killed by moderate subfreezing temperatures in less than 4 days was confirmed by this experiment. The incidental observation that larvae which have just attained the infective stage are readily killed by freezing was also confirmed.

OUTDOOR EXPERIMENTS WITH PREINFECTIVE LARVAE

In an experiment begun in February 1939, cultures originally containing large numbers of preinfective larvae were exposed for 14 to 71 days to outdoor temperatures. The total number of preinfective and infective larvae recovered from exposed cultures immediately on removal to the laboratory, or the number of infective larvae obtained from comparably exposed cultures incubated in the laboratory before examination, represented about 4.5 to 25 percent of the average yield of larvae from four unexposed control cultures, as shown in table 10. Two of the exposed cultures (Nos. 8 and 9) yielded almost as many larvae as one of the control cultures (No. 11). However, as a whole, the experiment indicated that the prevailing outdoor temperatures had a pronounced lethal effect on preinfective larvae. Culture 1, which was exposed 14 days, contained more surviving preinfective larvae than any of the test cultures examined after longer exposure. The preinfective larvae from this culture represented, however, only about 2.6 percent of the average number of larvae found in the control cultures. There was no definite correlation between increase in duration of exposure and increase in the number of infective larvae developing in exposed cultures. Therefore, the lethal action against preinfective larvae in this experiment undoubtedly occurred mainly during the first 14 days of exposure, from February 14 to 28. Apparently, the development of infective larvae also occurred mainly during this period. In one of the experiments with eggs, a pronounced lethal effect was also observed to occur during a 20-day period ending on February 28.

TABLE 10.—*Survival and development of free-living stages of horse strongyles in 50-gm. cultures of feces containing preinfective larvae when placed outdoors on Feb. 14, 1939*

Culture No.	Date culture was removed to laboratory	Duration of outdoor exposure	Range of temperature in each interval of exposure	Larvae recovered from culture when removed to laboratory			Infective larvae recovered after incubation of culture in laboratory for week or more	Apparent survival [1]
				Preinfective	Infective	Total		
	1939	*Days*	*°F.*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	Feb. 28	14	20-70	1,000	1,450	2,450		6.4
2							5,650	14.9
3	Mar. 17	30.7	23-70	500	6,000	6,500		17.1
4							4,600	12.1
5	Mar. 24	38	22-78	210	2,260	2,470		6.5
6							1,700	4.5
7	Apr. 7	52	29-78	0	4,500	4,500		11.8
8							8,200	21.6
9	Apr. 26	71	[2] 30-88	0	9,400	9,400		24.7
10	[3]						[4] 39,600	
11	[3]						[4] 10,450	
12	[3]						[5] 58,400	
13	[3]						[5] 43,700	

[1] Based on the average number of larvae recovered from the control cultures.
[2] Approximate, being temperatures recorded 1 mile away (thermometer used in experiment accidentally jarred).
[3] Unexposed; control.
[4] Examined March 22.
[5] Preinfective larvae; recovered when test cultures were exposed.

Some of the preinfective and infective larvae recovered from exposed cultures and some of the infective larvae which developed in exposed cultures incubated in the laboratory probably originated from eggs which had not yet hatched at the time that the feces were placed outdoors. Although this experiment provides evidence that, under the temperature conditions which prevailed, most of the preinfective larvae were killed before they reached infectivity, it is of equal importance that the fluctuating winter and spring temperatures occurring during a 71-day period did not "sterilize" feces originally containing mainly preinfective larvae of horse strongyles. At no time during this experiment was there an appreciable depth of snow on the ground.

DISCUSSION

The results of the writer's laboratory experiments on the survival of unembryonated eggs in feces continuously exposed to freezing are most nearly comparable to those reported by Enigk (*5*), since they showed that a large proportion of the eggs kept at mean temperatures of 14° to 21° F. (about −10° to −6° C.) were killed in 47 to 56 days. Since quantitative methods were used and the experiments were continued for longer periods than those reported by Enigk, the resistance of the eggs to continuous freezing has been better defined by the writer's work.

The reports of previous investigators do not afford a suitable basis for comparison with the results of the writer's laboratory experiments on the resistance of preinfective larvae to freezing. The absolute and comparative resistance of these larvae to moderately severe freezing has been determined by this investigation. De Blieck and Baudet's (*4*) observations afforded no comparison between the resistance of eggs and preinfective larvae. The experiments of

Wetzel (*13*) and Parnell (*10*) did not establish the absolute resistance of the preinfective larvae. Those of the latter worker permitted only a general comparison between their resistance and that of the other stages.

Experiments closely comparable to those reported in the present paper on the effect, on the eggs and preinfective larvae, of prolonged exposure to a mean temperature of about 36° F. (2° C.) also do not appear to have been reported by other investigators. As previously noted, Gackstatter (*6*) and De Blieck and Baudet (*4*) found that eggs were uninjured by brief exposure to similar temperatures. The writer's experiments appeared to show that even brief exposures killed some eggs.

The writer noted that first-stage and early second-stage larvae surviving prolonged exposure at about 36° F. had very pale intestinal cells. Probably larvae of these stages are not directly injured by temperatures a few degrees above the freezing point but die as a result of starvation. Late second-stage and noninfective third-stage larvae were able to continue their development to the infective stage at such temperatures. Thus, the survival of preinfective larvae was evidently conditioned by the degree of their dependence on bacterial food or, from a slightly different viewpoint, by their morphological status.

Parnell (*10*) and Britton (*3*) mentioned 10° C. (approximately 50° F.) as the approximate minimum temperature at which horse strongyle eggs and first-stage larvae can develop. The experimental basis for these statements was not specified by either writer. Wetzel (*13*) stated that the lowest temperature at which infective larvae of *Strongylus equinus* developed in about 350 hours was 12° to 13° C. (approximately 54° to 56° F.). About 10 percent of the hatched larvae became mature. However, in outdoor experiments of the writer's investigation, eggs became embryonated and apparently hatched in a period in which the maximum temperature did not exceed 46° F. (approximately 8° C.). Also, as previously mentioned, De Blieck and Baudet (*4*) noted that eggs kept for 12 days at 6° C. contained viable embryos. Hueber (*7*) reported that "sclerostome" eggs hatched in about 20 days at 5° to 10° C. The writer's observation that preinfective larvae reached infectivity during long exposure at a mean temperature of about 36° F. (maximum 50° F.) is not regarded as contradictory to Wetzel's report because greater time intervals were involved. Also, whether larvae of *S. equinus* were among those undergoing development was not determined.

The writer's incidental observation that third-stage larvae were not very resistant to subfreezing temperatures immediately after becoming infective presumably is in line with the statement of Zavadovskii and Vorob'eva (*14*) that the resistance capacity of *Strongylus equinus* larvae increases with age, 7-day-old larvae being more resistant to freezing than 4-day-old larvae. The English summary of these authors does not definitely state that the 4-day-old larvae were infective, however.

Parnell's (*9*) statement that embryonated horse strongyle eggs are much less resistant to freezing than unembryonated eggs has been confirmed. The data of this investigation do not permit comparison of the resistance of embryonated eggs and preinfective larvae.

In the light of the writer's experiments, Parnell's (*10*) statement that frequent alternate freezing and thawing can be relied on to kill

the free-living stages of horse strongyles requires qualification as does the previously quoted statements on this subject by Britton (*3*). The possible combinations of the variables temperature and time, as determinants of the lethal or nonlethal effect of alternations in freezing and thawing weather, are so numerous and complex that it is doubtful whether they can be reduced to a simple yet sufficiently inclusive verbal statement. The conditions stipulated by both writers were fulfilled in the outdoor experiments of the present investigation, but the free-living stages, including preinfective larvae, were not all killed and considerable numbers of infective larvae developed and survived. That alternate freezing and thawing weather may markedly reduce the potential yield of infective larvae in feces subjected to it is shown by this investigation. That suitable alternations are capable of causing virtually complete "sterilization" of feces originally containing eggs is also affirmed. But it is amply shown that the mere occurrence of frequent alternation of thawing and freezing weather affords no guarantee of sterilization.

Parnell's (*10*) later statement as to the susceptibility of all free-living stages to "violent and frequent fluctuations in temperature" differs somewhat from that just discussed. In the writer's experiments, infective larvae developed in feces originally containing eggs or preinfective larvae despite exposure to temperatures ranging from minima of 13° and 20° F., respectively, to a maximum of 88°, and despite repeated and rapid transitions from subfreezing to moderate temperatures.

With the exception of those reported in this paper, the only laboratory experiments known to the writer on the effects of alternate freezing and thawing on free-living stages of horse strongyles are those reported by Ober-Blöbaum (*8*), which involved infective larvae.

There is apparent disagreement between the findings of the present investigation and those of Parnell in the following respect: The writer found that large proportions of unembryonated eggs invariably were killed by continuous exposure to subfreezing temperatures for 7 to 8 weeks and that many evidently succumbed to much briefer exposure in laboratory experiments. Parnell (*9*), however, found that the continual low temperatures of a severe Canadian winter were insufficient to kill "sclerostome" eggs. Parnell's (*9*) statement that eggs exposed all winter embryonated and hatched normally in the spring suggests that none were killed. However, in the writer's opinion, the data of the experiment reported later by him (*10*) do not support such an interpretation.

The previously mentioned report of Baker, Salisbury, and Britton (*2*) appears to be more in agreement with the results of laboratory experiments, at least insofar as it tends to show that winter temperatures exert a lethal effect on horse strongyle eggs. However, although these authors reported the percentages of eggs killed by 65, 90, and 150 days of exposure in winter at Ithaca, N. Y., they did not include dates or temperature data, and the method of examination of the exposed feces serving as a basis for their calculations was not clearly described. For these and other reasons, it is difficult to evaluate the significance of the percentage relationships reported by these authors.

The writer's outdoor experiments did not show that winter temperatures near Beltsville had an appreciable lethal effect on horse strongyle

eggs as such. But the temperature conditions were very different from those of the laboratory experiments, which demonstrated that prolonged and continuous exposure to subfreezing temperatures kills the eggs. Results similar to those of these laboratory experiments would occur outdoors only in regions where subfreezing temperatures prevail continuously for long periods in winter and hence closely parallel the experimental temperature conditions.

The protection against extremes of temperature afforded to organisms on the soil surface by a covering of snow has generally been overlooked by those who have investigated the survival of the free-living stages of strongyles of horses and other domestic animals under winter conditions. Swales (*11*) found that, in the presence of an appreciable blanket of snow, temperatures recorded at the soil surface continuously approximated the freezing point and varied only slightly despite fluctuations in air temperature. He concluded that winter air temperatures cannot be considered as having any direct effect on organisms on the soil surface, so long as a covering of snow is present. The organisms are to be regarded as existing in a relatively constant climate with temperatures approximating the freezing point. The ground was covered with snow for a short time during only one of the three outdoor experiments reported in this paper.

In a discussion of the influence of frost on larvae in pastures, Taylor (*12*) stated that the cessation of the development of infective larvae during a cold period must result in the storage of potential infective material and, when warmer weather comes, a mass development of infective larvae probably occurs. Taylor referred to conditions under which grazing animals constantly deposit feces on a pasture during the cold period. However, the writer's experiments appear to indicate definitely that the proportion of eggs that ultimately yield infective larvae in horse feces exposed during cold seasons is determined by the particular relationships prevailing between the variables of temperature and time.

It is well known that the Baermann apparatus is of limited efficiency for the recovery of larvae from feces. However, it was used in this investigation because it is the most practicable available criterion of viability of such larvae. It was not determined whether the preinfective larvae recovered from feces following exposure were necessarily capable of completing their preparasitic development since it was impracticable to attempt to establish this point. Whether infective larvae developing in feces during or after exposure to low temperatures were capable of normal parasitic development also was undetermined. The writer is unaware of any method whereby this question might have been resolved satisfactorily.

More accurate mathematical results probably could have been obtained in laboratory experiments had counted numbers of eggs and preinfective larvae been exposed to low temperatures in water. But the significance of results obtained in this way would have been questionable. Feces are the natural medium of these stages for the most part, and the type of medium apparently affects experimental results. Thus, Zawadowsky and Vorobieva *(14)* found that infective larvae of *Strongylus equinus* showed greater resistance if frozen in damp sand or feces than if frozen in a dry condition or in water. The difficulty of determining whether larvae originating from eggs exposed in water could complete their development also would have

been introduced had this alternative medium been employed. Thus, it was felt, that despite the probability of variability in results, the use of feces, the natural medium, was preferable for the purposes of this investigation.

SUMMARY AND CONCLUSIONS

To determine the effects of continuous low temperatures, alternate freezing and thawing, and natural fluctuations in temperature in winter and early spring on the survival and development of horse strongyle eggs and preinfective larvae, laboratory and outdoor experiments were performed at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., in 1938 and 1939.

From 94 to 100 percent of unembryonated horse strongyle eggs in feces continuously exposed to mean temperatures of about 14° to 21° F. were killed in 47 to 56 days in laboratory experiments. The percentages of eggs surviving comparable exposures varied, and the absolute limit of the ability of the eggs to survive at the experimental temperature ranges was not established. In one experiment, 0.4 percent of the eggs survived 97 days of continuous exposure to subfreezing temperatures. The experiments suggested that the number of surviving eggs decreased gradually as the duration of exposure to subfreezing temperatures increased.

Embryonated eggs were much more readily killed than unembryonated eggs in feces kept at the same range of freezing and subfreezing temperatures (7°—32° F.). The absolute resistance of embryonated eggs was not determined.

The lethal effect on unembryonated eggs subjected to the following treatment was no greater than that obtained by continuous freezing: Repeated exposure to a mean temperature of 14° F., alternated with thawings at room temperature for periods only of sufficient duration to allow the feces to attain their normal consistency. When the thawings at room temperature were of sufficient duration to permit appreciable development, the cultures were promptly and almost completely sterilized so far as yielding infective larvae was concerned.

The limit of the ability of eggs to survive at a mean temperature of about 36° F. was not determined. In one experiment 0.5 percent survived an exposure of 195 days. In two of three experiments about one-half of the eggs survived for 3 to 4 weeks at this mean temperature, but because of variability in results, quantitative conclusions as to the rate of devitalization at this temperature were not warranted. Apparently, decrease in the number of surviving eggs and duration of exposure were related.

More than 90 percent of preinfective larvae principally in the first stage and early second stage of development were killed in 1.2 to 4 days in feces exposed to mean temperatures of 14°–21° F. Preinfective larvae in more advanced phases of development did not differ markedly from the younger larvae in ability to withstand subfreezing temperatures. Preinfective larvae in all phases of development were far more susceptible to subfreezing temperatures than the unembryonated eggs. Larvae that had just reached the infective stage were readily killed by subfreezing temperatures.

About 75 to 95 percent of preinfective larvae in cultures containing mainly first-stage and second-stage larvae survived at a mean tem-

perature of about 36° F. for periods up to approximately 2 weeks. About 25 to 50 percent survived for approximately 5 to 6 weeks. More than 90 percent died in about 3 months at this temperature, and those surviving prolonged exposure had very pale intestinal cells. Death of the larvae was attributed to starvation rather than to a direct injurious action of the temperature. The proportion of infective larvae in cultures originally containing principally larvae in the second ecdysis and advanced phases of the second stage increased markedly on prolonged exposure of the cultures at a mean temperature of about 36°. In these experiments the larvae surviving about 3 months of exposure represented 40 to 100 percent of the number of larvae of all stages originally present in the cultures.

When feces originally containing unembryonated eggs were exposed outdoors to winter and early spring temperatures in the vicinity of Beltsville, Md., some infective larvae developed during the winter and the feces were still capable of yielding additional larvae in the spring. One experiment was inconclusive as to the lethal effect of outdoor exposure, but in another experiment the number of infective larvae ultimately developing in exposed cultures represented about one-tenth of the number recovered from unexposed control cultures. Infective larvae also developed in winter in feces that contained preinfective larvae when exposed. The number of infective larvae that ultimately developed in exposed cultures or that such cultures were capable of yielding after exposure represented one-fourth to one-twentieth of the potential yield of the cultures. Eggs embryonated and hatched outdoors in a period in which the maximum temperature recorded was 46° F. During the outdoor experiments in which these results were obtained, the temperatures frequently fluctuated above and below the freezing point. Such fluctuations in winter and spring temperatures reduced the yield of infective larvae in exposed cultures but did not "sterilize" the cultures. The degree of the lethal effect of outdoor exposure in winter and spring depends on the relationships that prevail between the variables of temperature and time.

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INHERITANCE OF REACTION TO COMMON SCAB IN THE POTATO[1]

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INTRODUCTION

In the process of developing improved scab-resistant varieties of potatoes (*Solanum tuberosum* L.), it was found that individuals with similar clonal reactions often differed significantly in their breeding value. This led to an attempt to place the parental material into genetic groups based on the classification for scab reaction of their sexual progeny and to isolate the more desirable scab-resistant genotypes. The presentation includes four related series:

1. A study of a cross for the purpose of supplying a genetic classification that could be applied to the parental material. In this cross segregates and their sexual progenies from the F_1, F_2, and F_3 generations of a cross were utilized.
2. A study of parental material, consisting of 118 varieties or selections which were classified on the basis of the scab reaction of the sexual progeny into the same probable genotypes as were employed for the segregates from the above cross.
3. A study of the scab reaction of the hybrid progenies obtained by crossing certain of the above parental genotypes.
4. A study of the association between the color gene *P* and the reaction to scab.

MATERIAL

In 1924 a cross was made between Accession 123 and Lookout Mountain. Accession 123 was an unidentified clone obtained as a rogue in a field of Irish Cobblers. Both parents were self-fruitful, and the resulting hybrids were highly so. Under the plan of saving the seed in the breeding plots of all self-fruitful individuals, seed of a relatively large number of individuals from the F_1, F_2, and F_3 generations accumulated, though most of the individuals which produced this seed were discarded. When the studies on resistance to common scab were initiated, it was found that selections from this cross exhibited a wide range of scab reaction. Hence, this cross appeared to provide excellent material for a genetic classification of scab reaction. Recourse was then made to the seed which had accumulated from the hybrids of this cross during the previous 12 years. The segregates have each a pedigree number which shows their relationship and, by the number

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of numerals, the generation from which they were selected. Thus 5 is the cross number and is common to all the segregates. Similarly, 5–14 is an F_1 segregate whose progeny carries the same number, while 5–14–1, 5–14–2, 5–14–3 . . . are F_2 segregates from this progeny.

Parental material studied represented a cross-section of the self-fruitful clones in the breeding plots. It consisted of American and European varieties, and of selections from crosses and selfed lines. The pedigree numbers for the selections supply the same information as those previously indicated for the cross. The character of russeting was almost entirely absent in this parental material. Hence, the influence of russeting on reaction to scab as found in such varieties as Russet Burbank and Russet Rural was not considered.

METHODS

Relative resistance to common scab was determined by growing progenies in peat soil which was heavily infested with *Actinomyces scabies* (Thax.) Güss. as shown by the high degree of infection on certain individuals. Field tests were made in the first clonal generation except for one test in which certain progenies were retested in their second clonal generation. Another test was made by growing seedlings directly from seed on nonsterilized soil from the above field to which a single race of *A. scabies* was added. These progenies were tested in the field during the following summer.

Resistance was determined by the "highest scab" method as described by Leach et al.[2] According to this method, depth and severity of the scab lesion were used as a measure of susceptibility. Four degrees of severity were recognized, and these were indicated by numbers from 1 to 4 inclusive. The tubers of each individual of a given progeny were examined and the individual given a rating corresponding to the most severe type of pustule found. These ratings were then averaged to obtain the mean scab ratings. Each family was grown in two randomized blocks, and the interaction of families with blocks was used to obtain an estimate of the experimental error of the means.

It should be pointed out that the progenies used in this investigation were studied under limited conditions with respect to scab infection. Schaal[3] and Leach et al.[4] have shown that there are races of *Actinomyces scabies* which differ a great deal in pathogenicity on some lines of potatoes and that the same variety or seedling may differ in resistance when tested in different localities. Schaal[5] found that in general highly resistant varieties are consistently resistant when grown in widely different parts of the country, but that less resistant varieties may vary considerably. It was also pointed out by Wingerberg[6]

[2] LEACH, J. G., KRANTZ, F. A., DECKER, PHARES, and MATTSON, HAROLD. THE MEASUREMENT AND INHERITANCE OF SCAB RESISTANCE IN SELFED AND HYBRID PROGENIES OF POTATOES. Jour. Agr. Res. 56: 843–853, illus. 1938.

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that physiologic as well as morphologic factors may determine resistance to scab. Leach et al.[4] came to the same conclusion. This suggests the possibility that under a different set of conditions, especially with respect to the strains of the pathogen present, breeding behavior for resistance other than that reported in this paper may be found.

In the analysis of the data, cognizance was taken of the evidence supplied by Lunden [7] indicating that the potato was an autotetraploid. Hence, for a character conditioned by a single gene there would be five possible genotypes.

With this type of inheritance, the material did not appear to be sufficiently extensive to warrant testing more than a simple hypothesis. Therefore the analysis was made on the assumption that among the possible genes influencing scab reaction there might be one with a major influence which would allow a genetic classification of the material. The classification into the five possible genotypes designated as Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4 was made by inspection of the distribution of the progeny means for regions of discontinuity.

EXPERIMENTAL RESULTS

REACTION OF PROGENIES OF ACCESSION 123 × LOOKOUT MOUNTAIN

The sexual progeny of 14 F_1, 32 F_2, and 18 F_3 segregates of the cross Accession 123 by Lookout Mountain were tested for reaction to common scab. Their progeny means were then compared irrespective of generation for indications of the possible presence of five breeding types. The discontinuity in the distribution of the progeny means appeared to be greatest in four regions. A separation of the progeny means was made at these four regions, the segregates being divided into five groups. In table 1 is given the sexual progeny mean for each segregate and the distribution of the segregates in each generation among the five breeding types.

A comparison of the five breeding types is made in table 2, where the individual progenies of each type are combined and the distribution of the individuals from the progenies of each type among the classes of scab rating is shown. The distribution for each type is significantly different from that of the other types. This is indicated also by the differences between the type means and by the probability from χ^2 tests which is less than 0.01 in all cases.

This study, as previously stated, was undertaken on the hypothesis that a gene was present which had a major influence on scab reaction and that this would allow the classification of the breeding material into five genotypic classes. A comparison of the distribution of the segregates for each generation of the cross among the five types of breeding behavior with that of the distribution of the genotypes for each generation calculated on the assumption that the differences between breeding types was principally due to a single gene, can be made.

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[7] LUNDEN, AKSEL P. INHERITANCE STUDIES IN THE POTATO (SOLANUM TUBEROSUM L.). Norges Landbr HøiskolesÅrsmelding omÅkervekstforsøk (1934–35) [Title varies] 46: 1–156. 1937.

TABLE 1.—*Mean scab ratings of progenies of F_2 and F_3 segregates of a cross between Lookout Mountain and Accession 123* [1]

F_1 segregates		F_2 segregates		F_2 segregates		Breeding type
Designation of segregate	Progeny mean	Designation of segregate	Progeny mean	Designation of segregate	Progeny mean	
		5-3-1	0.67			1
5-3	1.17	5-14-6	1.00	5-4-14-1	1.17	
5-13	1.17	5-9-1	1.10	5-12-1-1	1.20	
		5-8-2	1.10	5-6-1-1	1.22	2
		5-2-1	1.11			
		5-10-3	1.19			
5-14	1.31	5-14-3	1.27	5-15-1-3	1.35	
5-16	1.32	5-10-1	1.27	5-10-1-1	1.37	
5-12	1.33	5-14-15	1.30	5-4-3-6	1.38	
5-10	1.40	5-7-2	1.33	5-10-1-2	1.47	
5-8	1.42	5-12-1	1.33	5-10-3-2	1.48	
5-9	1.42	5-8-1	1.36	5-10-1-3	1.55	
5-7	1.49	5-14-16	1.38	5-11-1-3	1.59	
5-1	1.50	5-2-5	1.40	5-14-5-1	1.63	
5-5	1.50	5-11-1	1.40	5-11-1-4	1.80	
5-11	1.76	5-15-1	1.50	5-10-1-5	1.89	
		5-3-2	1.63			3
		5-6-1	1.65			
		5-14-8	1.68			
		5-14-1	1.74			
		5-14-17	1.77			
		5-2-4	1.80			
		5-2-2	1.80			
		5-14-2	1.81			
		5-10-2	1.82			
		5-4-3	1.85			
		5-14-7	1.87			
5-4	1.92	5-4-1	2.05	5-14-8-1	1.96	
5-2	1.98	5-6-2	2.21	5-4-1-1	2.00	
		5-14-5	2.27	5-4-3-4	2.21	4
		5-13-1	2.29			
		5-1-1	2.35			
				5-4-3-5	2.45	
				5-10-1-4	2.47	

[1] 2×S. E. of a difference=0.38.

TABLE 2.—*Distribution of the individuals of the progenies of each breeding type*

Breeding type	Number of progenies	Number of individuals in the progenies of each type falling into the indicated classes of scab rating [1]					Mean	*P* from χ^2 test between types
		0	1	2	3	4		
1	1	6	8	1	0	0	0.67	
2	10	18	173	44	4	0	1.13	1 and 2 less than 0.01.
3	42	46	598	377	105	19	1.52	2 and 3 less than 0.01.
4	11	8	77	125	94	17	2.11	3 and 4 less than 0.01.
5	2	2	15	13	19	15	2.47	4 and 5 less than 0.01.

[1] 0=No scab, 4=most severe scab.

The 14 F_1 segregates are distributed among breeding types 2, 3, and 4. If the 5 types are represented by the following genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4, then in the F_1 2 segregates would be classed as triplex, 10 as duplex, and 2 as simplex. A similar distribution in the F_1 might be obtained if 1 parent, Accession 123, was triplex and the other parent, Lookout Mountain, was simplex for the gene *Sc*. A comparison of the obtained with the calculated distribution is shown in table 3.

This assumption agrees with the information available concerning the parents, which unfortunately is meager since both were discarded before the scab studies were undertaken. Hybrids obtained from

other progenies with Lookout Mountain have given progenies with very little resistance. On the other hand, selfed lines derived from Accession 123 have shown high resistance, some of them approaching that of Hindenburg selfed. Accession 123 was not homozygous since there was a significant difference in scab reaction between some of the selfed lines derived from it.

TABLE 3.—*A comparison of the obtained with the calculated genotype distribution in the F_1 segregates of the cross Accession 123 × Lookout Mountain*

Distribution—	Sc_1	Sc_2sc	Sc_2sc_2	$Scsc_3$	sc_4
Obtained	0	2	10.0	2	0
Calculated	0	3.5	7.0	3.5	0

The theoretical distribution which would be obtained in the F_2 and F_3 can be calculated on the basis that a random sample was taken from each generation. The segregates probably represented a random sample, for they included all of the self-fruitful plants in each generation. Unfortunately only a selected group was carried into each succeeding generation. However, the breeding plots were relatively free of scab, and no consideration was given to scab reaction in selecting the segregates for the succeeding generation. In table 4 the distribution is calculated on a chromosome rather than a chromatid type of segregation as the former is in closer agreement with the obtained distribution.

TABLE 4.—*A comparison of the obtained with the calculated genotype distribution in the F_2 and F_3 segregates of the cross Accession 123 × Lookout Mountain*

[Calculations made on a chromosome rather than a chromatid type of segregation]

Distribution of—	Sc_1	Sc_2sc	Sc_2sc_2	$Scsc_3$	sc_4
F_2 segregates:					
Obtained	1	5	21	5	0
Calculated	1.2	7.2	15.2	7.2	1.2
F_3 segregates:					
Obtained	0	3	10	3	2
Calculated	2	4	6	4	2

REACTION OF PROGENIES FROM OTHER VARIETIES AND SELECTIONS

The reaction to common scab of selfed progenies from 118 other varieties and selections have been studied. Tests were made in the field in 1936, 1937, 1938, and 1939 and in the greenhouse during the fall and winter of 1938–39. Since the level of infection varied in the different tests, each comparable group is listed separately in table 5.

These varieties and selections have been classified into five breeding types. In making this classification consideration has been given to: (1) Regions of discontinuity in the progeny means; (2) the reaction of progenies which were tested under more than one level of infection; (3) the reaction of the self lines from a variety or selection.

It will be noted in table 5 that the variety Hindenburg is the sole individual classified in breeding type 1. Its sexual progeny mean in the four trials in which it was tested was consistently low. This variety does not appear to be homozygous for all factors which influence scab reaction since two of its segregates, 14–6 and 14–7, gave a significantly higher progeny mean in the two trials in 1939.

TABLE 5.—*Mean scab ratings of selfed progenies from varieties and selections 1936–39*

1936		1937		1937		1938		1939			Breeding type
Variety or selection	Progeny mean [1]	Variety or selection	Progeny mean [2]	Variety or selection	Progeny mean [1]	Variety or selection	Progeny mean [1]	Variety or selection	Progeny means		
									Greenhouse [3]	Field [1]	
Hindenburg	1.10	Hindenburg	1.29					Hindenburg	1.19	1.74	1
11–1–25	1.00	11–1–25	1.67					HC15	1.21	2.13	2
1–1	1.07	1–1	1.79					Jubel	1.26	2.33	
1–4–1	1.37	1–4–1	1.81					5–10–1–29	1.27	2.19	
15–1	1.19							5–2–8	1.40	2.27	
82–11	1.16	82–11	2.05	22–1	1.43	11–1–2–2–4–3	1.89	5–15–1–4–1–4	1.33	2.64	
11–8–1–9–4	1.22	11–8–1–9–4	2.19	3–4	1.46	11–1–2–2–4–2	1.94	124.35–2	1.62	2.60	
11–1–2–2–4	1.21	11–1–2–2–4	2.22	17–2	1.59	5–4–1	2.07	14–7	1.62	2.93	
5–10–1	1.21	5–10–1	2.52	11–9–1–8–1	1.70	1–1–6	2.11	5–10–1	1.30	3.15	
21–2–2	1.12	21–2–2	2.55	4–9–3–1–1–1–1–1	1.78	5–14–8–1–1	2.11	5–14–8–1–1	1.21	2.75	
29–13–5	1.22			4–11–1–3–3	1.87	12–2–4–1–7–3	2.13	41–1–1–7–6–1	1.62	3.06	
11–8–1–9–3	1.27			5–15–1–4–1–2	1.85	5–15–1–4–1–2	1.67	14–6	1.65	2.40	
11–1–2–1–1	1.29					15–2	2.22	5–10–3–23–4	1.69	2.81	3
						5–10–1–19	2.25	15–2	2.15	2.81	
						5–10–1–17	2.58	11–6–1–1–1–1–1	1.78	2.96	
						5–14–8–1–2	2.58	17–2–2	1.80	3.12	
						16–1	2.59	17–2–1	1.84	2.50	
						4–25–6	2.63	165.35–1	1.86	2.37	
						11–8–1–9–4	2.66	5–10–3–4	2.00	2.20	
						15–1–2	2.69	5–10–3–14	1.86	2.19	
								5–4–1–1–7	2.10	2.50	
82–8	1.35			11–8	2.17	41–1–1–7–5–1	2.72	66–6–2	1.45	3.26	
82–6	1.35			5–15–1–4–1	2.24	11–1–2–2–4–1	2.77	9–1–2	1.74	3.16	
19.33–1	1.36			30–11–3–1	2.36	15–1–1	2.93	5–4–1–1–3	1.82	2.73	
10–4–2	1.36					39–3–1–1–1–1	3.03	11–8–1–9–1–4	1.84	3.35	
5.33–1	1.38										
40–2–2	1.39					41–2–10–1	3.06	11–8–1–9–3–1	1.84	3.06	4
82–4	1.40					82–1–1	3.07	66–6–3	1.97	2.80	
12–2–4–1–7	1.43	12–2–4–1–7	2.63					66–5–1	1.97	3.06	
82–10	1.44							Earlaine	2.00	2.69	
11–8–1–9–1	1.45							5–14–8–18	2.06	2.85	
11–1–2–1	1.51							5–10–3–23–2	2.19	3.04	
1–4–2	1.54							5–10–1–42	2.60	2.69	
34–1–1	1.62					34–1–1–1	3.09	124.35–1	2.00	3.34	
82–2	1.63					27–1	3.32	66–69–1	1.78	3.52	
82–1	1.64					82–4–1	3.33	11–1–2–3–2	1.86	3.40	
3–2–4	1.66					4–25–7–1	3.38	80–7–5	2.16	3.40	

29-14	1.80					13-1	3.46	Chippewa	2.30	3.30
81-1	2.00					21-2-2-11	3.50	80-7-1	2.37	2.87
29-13	2.03					5-14-8-1-7	3.53	60-6-1	2.39	
11-1-3-2	2.10	11-1-3-2	2.91			13-1-1	3.60	66-4-1	2.45	3.22
						11-1-3-2-18	3.67	5-4-1-1-4	2.70	3.73
						80-11-3	3.70	80-7-6	2.71	3.53
						11-1-3-2-11	3.70	80-7-3	2.75	3.06
						11-1-25-3	3.71	80-7-2	2.79	3.42
						11-1-3-2-14	3.82	80-7-4	2.88	3.57
						11-1-3-2-20	4.00			
2 × S. E. of difference.	0.43		0.33		0.38		0.46		0.35	0.54

[1] First clonal generation. [2] Second clonal generation. [3] Seedling generation.

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In breeding type 2 are eight individuals, including the variety Jubel. The clonal reaction of these eight individuals is very similar to that of Hindenburg. Four of these individuals, 11–1–25, 1–1, 1–4–1, and 15–1, produced sexual progenies whose mean scab reaction was not significantly different from that of the progeny of Hindenburg in 1936. In the second clonal generation in 1937 the sexual progenies of three of these individuals appeared to give a significantly higher scab reaction than that of the progeny of Hindenburg. The remaining four individuals of breeding type 2 gave sexual progenies with a small but consistently higher scab reaction in both the greenhouse and the field test than that of Hindenburg in 1939.

Forty-two clones are classified in breeding type 3. The position of this group was marked by including comparable sexual progenies of selection 5–10–1 in five of the six tests. The limits of the group were determined by separation in the regions of the greatest discontinuity in the distribution of the progeny means. The separation in 1937 is the same as that made for the segregates of the cross given in table 1 since these were included in the same test. The trial in 1938 did not include the progeny of selection 5–10–1. However, this trial included the progeny of selection 5–15–1–4–1–2, of which a comparable sexual progeny was tested in 1937, and the progeny of selection 15–2, from which a similar sexual progeny was tested in the greenhouse and in the field in 1939. The sexual progenies of individuals classified in breeding type 3 appeared to be more variable than those from the other breeding types. Thus the sexual progeny of selection 5–10–1 gave a scab reaction similar to that of the individuals placed in breeding type 2 in 1936. Under a higher level of infection in the second clonal generation tested in 1937, this sexual progeny approached breeding type 4. Again in 1939 in the greenhouse test the progeny mean was relatively low, approaching that of the sexual progeny of the variety Jubel, and was significantly lower than the progeny of the variety Earlaine. In the field tests which followed a higher level of infection was obtained, and the progeny mean of selection 5–10–1 is significantly higher than that of the progeny of Hindenburg and Jubel and approaches the progeny mean of Earlaine. This indicated difference in reaction to high and low levels of infection in the tests has been considered in the classification of the individuals into breeding type 3. A similar difference in reaction to low and high levels of infection has been noted in the clonal reaction of this group. Their clonal resistance to scab is sufficient to be of commercial value under ordinary levels of infection.

In breeding type 4 are grouped 29 clones including the variety Earlaine. The variety Katahdin also probably belongs to this group since selections from its selfed progenies (line 66) are included. This group differs from breeding type 3 in having slightly higher sexual progeny means. However, their progeny means are significantly lower in general than those of breeding type 5. The latter are considered to carry no resistance. In clonal tests, individuals in breeding type 4 were distinguishable from those of type 3 but not from those of type 5. While more intensive studies will probably indicate the presence of a greater clonal resistance in the individuals belonging to breeding type 4 than those of type 5, it would hardly be sufficient to be of commercial value.

Thirty-five clones including the variety Chippewa are grouped in breeding type 5. This group shows no clonal resistance to scab. Attempts to isolate resistant individuals from their sexual progenies have met with no success. A few of the more promising isolates were given progeny tests. Thus it will be noted that the progenies of 11–1–3–2–(11) (14) (18) and (20), all selections from 11–1–3–2, gave high progeny means. Similar selections from line 80–7 gave high progeny means as will be seen from the progeny means of selections 80–7–(1) (2) (3) (4) (5) and (6).

REACTIONS OBTAINED IN CROSSING PARENTAL TYPES

The reaction to scab of the crossed progenies as obtained in the field in 1936 and 1938 and in the greenhouse and field in 1939 are presented in table 6. The parents employed were previously classified from the reaction of their selfed progenies with the exception of the varieties Early Ohio, Triumph, and Warba. The crosses are listed in the order of their mean scab reaction.

In 1936, crosses between parents of breeding types 2, 3, 4, and 5 were tested. The two crosses of type 2 (Jubel) by selections of type 3 and 4 gave the lowest progeny mean. The two crosses between parents of type 5 gave the highest progeny mean. The breeding behavior of the varieties Early Ohio, Triumph, and Warba appears to be the same. From the progeny means of the crosses in which these varieties enter, they are classified as probably belonging to breeding type 5.

Crosses between the same breeding types as were tested in 1936 were tested in 1938. The progeny means of these crosses are in fair agreement with the classification made from their selfed progenies with the possible exception of selection 80–7. This parent was classified in breeding type 5 on the basis of the high mean scab reaction of its selfed lines. Its behavior in these crosses suggests that it may carry some factors which influence low scab reaction.

The results with the crosses tested in the greenhouse and field in 1939 emphasize the low progeny means obtained when Hindenburg enters into a cross. The cross of type 2, (Jubel) by type 3, selection 5–10–1, gave a somewhat higher scab reaction than the average of the four crosses in which Hindenburg was crossed with selections of type 4. In the 1936 and 1938 tests, which contained no crosses with Hindenburg, the same cross, Jubel with 5–10–1, gave the lowest average mean scab reaction. Clark et al.[8] from observations on two F_1 progenies concluded that Hindenburg was probably homozygous for resistance. The mean scab reaction of the selfed progeny of Hindenburg given in table 5 and its behavior in the crosses as shown in table 6 suggest that this variety is nearly homozygous for scab resistance. However, two selections, 14–6 and 14–7, from the selfed progeny of Hindenburg, produced selfed progenies with a significantly higher mean scab reaction than their parent progeny (see table 5). This suggests that it is heterozygous for at least some minor factors which influence the mean scab reaction. The results of the 1939 test also supply further evidence that the varieties Triumph and Early Ohio probably carry no factors influencing low scab reaction.

[8] CLARK, F. C., STEVENSON, F. J., and SCHAAL, L. A. THE INHERITANCE OF SCAB RESISTANCE IN CERTAIN CROSSES AND SELFED LINES OF POTATOES. Phytopathology 28: 878–890, illus. 1938.

TABLE 6.—*Mean scab ratings of hybrid progenies from certain breeding types as determined in the field in 1936 and 1938 and in the field and greenhouse in 1939*

1936 TEST

Parent	Breeding type	Parent	Breeding type	Number of seedlings	Progeny mean	
					Greenhouse	Field
Jubel	2	× 5-14-8-1	4	15		1.00
Jubel	2	× 5-10-1	3	15		1.07
4-9-3-1-1-1-1-1	3	× 82-10	4	44		1.21
4-9-3-1-1-1-1-1	3	× 5-10-1	3	68		1.26
Early Ohio	5	× 4-9-3-1-1-1-1-1	3	11		1.27
Triumph	5	× 5-10-1	3	104		1.39
Warba	5	× 5-10-1	3	59		1.40
11-1-2-2-4	3	× 21-2-2	3	31		1.42
4-25-6	3	× 5-10-1	3	26		1.42
82-10	4	× 12-2-4-1-7	4	109		1.42
Early Ohio	5	× 5-10-1	3	34		1.44
11-8-1-9-4	3	× 21-2-2	3	67		1.46
Warba	5	× 12-2-4-1-7	4	17		1.59
Early Ohio	5	× 12-2-4-1-7	4	23		1.74
Warba	5	× 11-1-3-2	5	32		2.00
Triumph	5	× 4-25-7-1	5	26		2.38
2 × S. E. of difference						.43

1938 TEST

Jubel	2	× 5-10-1	3	47		2.08
30-11-3-1	4	× 5-10-1-29	2	22		2.73
17-2	4	× 5-10-1	3	69		2.85
11-8-1-9-3	3	× 80-7	5	22		2.95
82-11	4	× 80-7	5	37		2.97
17-3	3	× 5-10-1	3	81		3.01
15-2	3	× 80-7	5	72		3.04
80-7	5	× 5-10-1	3	61		3.08
80-7	5	× 15-2	3	51		3.18
13-1	5	× 80-7	5	36		3.25
11-8-1-9-3	3	× 5-10-1	3	24		3.31
21-2-2	3	× 5-10-1	3	44		3.35
15-2	3	× 5-10-1	3	17		3.35
82-6	4	× 15-2	3	30		3.37
82-6	4	× 5-10-1	3	47		3.38
13-1	5	× 5-10-1	3	43		3.51
11-1-3-2	5	× 5-10-1	3	28		3.52
13-1	5	× 11-1-3-2	5	28		3.75
2 × S. E. of difference						.46

1939 TEST

39-3-1-1-1-1	4	× Hindenburg	1	75	1.37	2.16
Hindenburg	1	× 5-14-8-1	4	69	1.65	2.21
41-1-1-7-5	4	× Hindenburg	1	43	1.44	2.40
Jubel	2	× 5-10-1	3	80	1.38	2.42
5-10-3-4	3	× 15-2	3	70	1.46	2.46
Triumph	5	× Hindenburg	1	42	1.68	2.53
82-8	4	× Hindenburg	1	80	1.38	2.67
5-15-1-4-1-2	3	× 11-1-25	2	88	1.75	2.72
HC 15	2	× 5-14-8-1	4	93	2.37	2.85
13-1	5	× Hindenburg	1	84	1.51	2.86
13-1	5	× 5-10-1	3	67	1.36	2.92
Triumph	5	× Jubel	2	70	1.39	2.95
11-1-3-2-18	5	× 5-10-1	3	10	2.14	3.00
Early Ohio	5	× 5-14-8-1	4	37	2.05	3.50
Triumph	5	× 5-14-8-1	4	70	2.30	3.39
5-10-3-4	3	× 11-1-3-2-18	5	54	2.68	
2 × S. E. of difference					.35	.54

ASSOCIATION BETWEEN THE *P* FACTOR AND RESISTANCE TO SCAB

The possible association between the plant color factor P and scab resistance was studied in 13 crosses. A susceptible P_{p_3} clone was crossed with the p_4 clone 5–10–1, which is classified as of breeding type 3. The P_{p_3} plants from the F_1 were then crossed with p_4 plants. The results are presented in table 7. The separation of the P and p plants is easy and accurate. By having the red color factors, either E or R, present, the plants with p have red and the plants with P have purple tubers. It will be noted that the plants with purple tubers (P) had a higher mean scab rating in all the crosses except one. The differences in mean scab rating are too small to be significant except in a cross of Russet Rural by 5–10–29. When all the crosses are considered the differences become highly significant.

TABLE 7.—*Mean scab ratings of P and p plants in 13 crosses*

Parents of cross		Plants with purple tubers (P)		Plants with red tubers (p)	
P_{p_3}	p_4	Number	Mean scab rating	Number	Mean scab rating
76–1	× 5–10–29	7	2.00	17	1.47
77–8	× 5–10–29	18	1.78	15	1.07
77–9	× 5–10–29	42	2.62	37	1.95
76–1	× 11–1	14	2.05	9	2.00
77–8	× 11–1	9	1.89	11	2.00
77–9	× 11–1	21	2.42	30	2.39
76–1	× 12–2	20	3.21	6	1.50
77–8	× 12–2	15	2.40	11	1.64
77–9	× 12–2	17	2.59	13	2.48
77–8	× 75–5	30	1.80	30	1.73
Russet Rural	× 5–10–29	45	2.73	27	1.93
Do	× 5–10–1	29	3.50	48	2.97
Do	× 5–14–8–1	14	3.36	6	2.80
Total or mean of 13 families		281	2.49	260	1.99

DISCUSSION

The autotetrasomic type of inheritance in the potato limited the study of classifying the varieties and selections to one based on the hypothesis that the reaction to scab was influenced sufficiently by one gene to allow a grouping of the material into the five possible genotypes. In classifying the segregates of the cross between Accession 123 and Lookout Mountain, the principal reliance was placed on the means of their sexual progenies. For in interpreting the results of progeny tests, it is necessary to remember that scab is a disease caused by a pathogen comprising many physiologic races and that the extent of infection is influenced by factors such as temperature, moisture, pH, and the stage of maturity of the plant. The use of the type of pustule, rather than the extent of scabbed surface, reduces the variability caused by these factors to some extent, but the variation is still too large to utilize a phenotypic classification of the individuals within the progenies.

Assuming that at least five genetic groups were present, the segregates of the cross between Accession 123 and Lookout Mountain were separated in the regions of the greatest discontinuity of their sexual progeny means into five groups designated as breeding types. The

distribution of individuals within the sexual progenies of each breeding type was found by the χ^2 test to be significantly different from any of the other types.

The five breeding types were assumed to represent the following genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4; and the parent genotypes were assumed to be, for Accession 123, Sc_3sc, and for Lookout Mountain $Scsc_3$. The observed distribution in the F_1, F_2, and F_3 was found to be in fair agreement with that calculated from the above hypothesis. This study appeared to justify an attempt to classify the breeding material from the scab reaction of their sexual progeny into breeding types. In making this classification, consideration was given to regions of discontinuity in the progeny means, to reactions of sexual progenies tested under more than one level of infection, and to the reaction of the sexual progeny of the segregates from a selfed line of a variety or selection. Further studies will undoubtedly suggest shifting some varieties and selections to groups different from those in which they are now located. These groups are not considered to be homogeneous, for the reactions to scab obtained from isolates of the sexual progenies of individuals classified in breeding types 1 and 5 indicate that these individuals are probably not homozygous for all factors influencing scab reaction. The results obtained from crosses between types agree reasonably well with the reactions to scab of the selfed progenies. The data indicate that testing varieties and selections by crossing would be of value when large differences are present. The data also supplied information on the breeding behavior of the Early Ohio, Triumph, and Warba varieties from which selfed seed was not available. The association obtained between the *P* factor and reaction to scab suggests that a major factor is present in this chromosome group, for as the experiment was set up, only the influence of a gene or possibly a number of genes in the simplex condition was measured.

SUMMARY

Starting with the assumption that the type of inheritance in the potato was autotetrasomic and that the difference observed in reaction to common scab was principally due to the influence of one gene, the segregates obtained from the F_1, F_2, and F_3 generations of a cross between Accession 123 and Lookout Mountain were classified into five breeding types. The separation was made in regions where the mean scab reaction of the sexual progenies showed the greatest discontinuity. The distribution of the segregates of the five breeding types in the F_1, F_2, and F_3 generation approached that calculated on the hypothesis that Accession 123 was triplex and Lookout Mountain was simplex for a gene *Sc* influencing reaction to scab and that the five breeding types corresponded to the genotypes Sc_4, Sc_3sc, Sc_2sc_2, $Scsc_3$, and sc_4.

One hundred and eighteen varieties and selections of heterogeneous origin were classified from sexual progeny tests into five groups corresponding to the above breeding types as follows:

Type 1. The variety Hindenburg.
Type 2. Seven selections and the variety Jubel.
Type 3. Forty-two selections.
Type 4. Thirty-one selections and the variety Earlaine.
Type 5. Thirty-four selections and the Chippewa variety.

Hindenburg, which was classified in type 1, gave a progeny from which two segregates were isolated whose sexual progeny gave a significantly higher mean scab reaction than the progeny of Hindenburg.

Crosses between types gave progenies whose mean reaction to scab was in general agreement with the reaction of the selfed progenies of the parents. The Early Ohio, Triumph, and Warba were classified in type 5, from the mean scab reaction of the crossed progenies, obtained when these varieties were used as female parents.

An association between the color factor P and the mean scab reaction was observed in a study of 13 crosses. The mean scab reaction of the P and p plants for the 13 crosses was 2.49 and 1.99 respectively.

USE OF THE PENETROMETER FOR DETERMINING THE FIRMNESS OF FATTY TISSUE OF HOG CARCASSES [1]

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INTRODUCTION

An accurate, moderately rapid, readily applied, and inexpensive method for determining the firmness of fatty tissue of chilled meat-animal carcasses would be of value to research workers concerned with the quality of meat, especially pork, in which there is much variation in firmness. Furthermore, it is possible that such a method would be useful under commercial conditions.

Several methods have been used for determining the firmness of animal fats or for determining the factors closely related to firmness. Among them are the manual test involving human judgment, the refractive index, iodine number, and the melting point. The manual test, as developed and applied by the Bureau of Animal Industry to the fatty tissue of hog carcasses, provides for the use of five degrees, or grades, of firmness—hard, medium hard, medium soft, soft, and oily. The chief objection to this method is the error introduced by the human factor. The error may be reduced considerably by having a committee of qualified judges. This is customary in research but is seldom done in commercial work. It has been shown [3] that a close relation exists between grade of firmness and refractive index, as well as between the former and iodine number. In the case of melting point, the relation was found to be less close. Other analyses have substantiated these findings. Values for 200 samples, representing 40 each of the 5 grades of firmness, gave the following coefficients of correlation: [4] Firmness grade and refractive index, -0.900 ± 0.004; firmness grade and iodine number, -0.899 ± 0.009; firmness grade and melting point, $+0.788 \pm 0.018$; refractive index and iodine number, $+0.960 \pm 0.004$.

Since there is little difference between refractive index and iodine number as a measure of firmness, the former has been extensively used by the Bureau as a routine laboratory test because of the greater rapidity and economy with which it can be determined. However, this method, as well as the iodine-number and melting-point tests, requires relatively expensive laboratory facilities and is rather time

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[2] Acknowledgment is made to Mrs. Edna V. Steely for assistance in the statistical calculations.

[3] HANKINS, O. G., and ELLIS, N. R. SOME RESULTS OF SOFT-PORK INVESTIGATIONS. U. S. Dept. Agr. Bul. 1407, 68 pp., illus. 1926.
—— ELLIS, N. R., and ZELLER, J. H. SOME RESULTS OF SOFT-PORK INVESTIGATIONS, II. U. S. Dept. Agr. Bul. 1492, 50 pp., illus. 1928.

[4] The refractive indexes, iodine numbers, and melting points of these samples were determined by N. R. Ellis of this Bureau.

consuming. Consequently an investigation of the possible merit of an instrument known as a penetrometer for measuring the firmness of chilled fatty tissue was believed to be desirable. This instrument has been used by scientific and commercial agencies with considerable success to determine the firmness of certain foods, such as cakes and jellies, by measuring the depth that a needle of specific size and bluntness will penetrate under a standard load during a specific time. It was felt that the manual test for firmness and the penetrometer determination involve the same principle. The objects of the study reported in this paper were to determine the usefulness of the penetrometer for measuring the firmness of fatty tissue of chilled hog carcasses and to establish tentative grade limits in terms of penetrometer readings in order that individual carcasses may be classified with respect to firmness.

EXPERIMENTAL PROCEDURE

Fat samples from the carcasses of 188 hogs slaughtered in the spring and fall of 1938 and the spring of 1939 in various projects conducted at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., were used in the present experiment. After being chilled for approximately 72 hours at a temperature of 33° to 35° F. (0.56° to 1.67° C.), the carcasses were graded for firmness by a committee of three qualified judges. The designated grades were hard, medium hard, medium soft, soft, and oily. The range in firmness of the carcasses was too narrow to provide good representation for all 5 grades. One of the writers, therefore, visited a commercial packing house in the spring of 1939 and selected 163 carcasses from which supplementary samples were obtained. He graded the carcasses and, on returning to Beltsville, graded the 163 samples with the assistance of 2 other experienced men. From the 2 sources mentioned, samples were procured from carcasses graded as follows: 116, hard; 70, medium hard; 49, medium soft; 65, soft; and 51, oily.

In each instance the sample of fatty tissue was removed from the carcass along the middle of the back according to the method described by Hankins and Ellis.[5] The skin was then removed, and the sample was squared and trimmed to the proper length to fit the sample box, which had inside dimensions of 3 by 3 by 1 inches. Immediately prior to testing, a thin slice was removed from the median side of the fat sample in order to provide a fresh, undried surface, and the sample was placed in the box with the surface upward. Figure 1 shows a sample ready to be placed in the box and another sample in the box ready for testing. No cover or weight was used to hold the sample in place; to keep it from slipping sideways in the box, pieces of fatty tissue were packed around it. Care was exercised not to put, on the sides of the sample, any pressure that might influence the depth of penetration of the needle.

The penetrometer needle used was 3 inches long with the working end 0.15 inch in diameter. The rounded point represented a radius of 0.075 inch. The total weight of the needle, the test rod that held it, and added load was 255.65 gm. With the aid of the mirror attached to the penetrometer the technician adjusted the working unit, consist-

[5] HANKINS, O. G., and ELLIS, N. R. See footnote 3.

ing of the needle, test rod, and added load, so that the point of the needle barely came in contact with the sample. The weight of 255.65 gm. was then allowed to force the needle into the sample for 15 seconds. Three penetration tests were made on the outer layer of back fat and three tests on the inner layer, and the average of the six determinations was recorded as the value for the sample as a whole. Penetration was determined in tenths of a millimeter and at a sample and

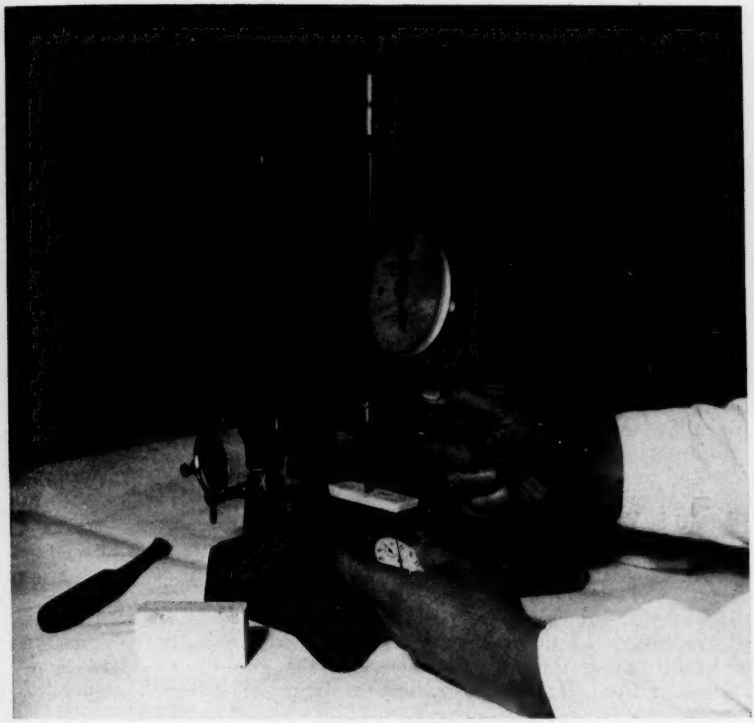

FIGURE 1.—Penetrometer used in making determinations on the fatty tissue. A sample is being tested, with another sample, at the left, ready for testing.

laboratory temperature within the range of 33° to 35° F. (0.56° to 1.67° C.).

After the penetrometer tests had been made the samples were rendered at 230° to 239° F. (110° to 115° C.) and filtered. Refractive-index values of the fat were then determined at 104° F. (40° C.).[6]

USEFULNESS OF PENETROMETER FOR DETERMINING FIRMNESS

It is shown in table 1 that as firmness decreased from hard to oily the depth of penetration of the needle increased for both the outer and inner layers of back fat. On an average, for the two layers

[6] The refractive-index values for study in relation to penetrometer determinations and firmness grades were determined by Willard E. Folland of the Bureau.

there was a difference of 60.3 units, or 6.03 mm., between the greatest, and least depths of penetration. For the hard and medium-hard grades there was slightly greater penetration into the outer layer than into the inner layer of back fat. On the other hand, for the medium-soft, soft, and oily grades, the greater penetration was into the inner layer.

TABLE 1.—*Mean, range, and standard deviation of penetrometer determinations*[1] *and refractive indexes of fat samples representing five degrees of firmness*

Committee firmness grade (manual test)	Number of samples	Penetrometer determinations, in tenths of a millimeter					Refractive index		
		Outer layer of back fat	Inner layer of back fat	Average of outer and inner layers of back fat	Range[2]	Standard deviation[2]	Average of outer and inner layers of back fat	Range[2]	Standard deviation[2]
Hard	116	20.8	16.4	18.6	10–42	5.3	1.4595	1.4589–1.4602	0.0003
Medium hard	70	27.6	24.6	26.1	11–45	8.1	1.4601	1.4592–1.4611	.0004
Medium soft	49	37.9	44.9	41.4	19–59	9.6	1.4607	1.4595–1.4615	.0005
Soft	65	46.4	74.0	60.2	29–96	14.4	1.4615	1.4598–1.4631	.0006
Oily	51	56.4	101.4	78.9	48–109	11.7	1.4622	1.4616–1.4632	.0003

[1] Conditions of experiment: Radius of point of needle, 0.075 inch; total weight of needle and added load, 255.65 gm.; period of penetration of needle, 15 seconds.
[2] Represents values for average of outer and inner layers of back fat.

Table 1 also shows for each firmness grade the range and standard deviation of the penetrometer determinations. Similar data are given for refractive indexes. Between the medium hard and hard grades, medium soft and medium hard, soft and medium soft, and oily and soft, respective differences of 7.5, 15.3, 18.8, and 18.7 units in depth of penetration were found. The corresponding differences in refractive index were 0.006, 0.006, 0.008, and 0.007.

Also it is shown in table 1 that within each of the five groups of samples, classified according to committee grade for firmness, considerable variation occurred in the depth to which the needle penetrated. Likewise, rather wide variation in refractive index within the grade is indicated. Figure 2 shows graphically the variation of penetrometer determinations within the grade. Standard deviations for the soft and oily grades are larger than those for the hard grades.

A calculation of coefficients of correlation for data obtained by a committee of judges using the manual test for firmness, the penetrometer determinations, and the refractive-index values showed the closest relation between the committee grade for firmness and refractive index, the coefficient being -0.917 ± 0.006. The next highest coefficient was -0.905 ± 0.007, representing the correlation between committee grade for firmness and penetrometer determination. The negligible difference between these relationships indicates that the refractive index or penetrometer determination may be used with equal satisfaction in measuring firmness. Between refractive index and penetrometer determination the correlation was $+0.878 \pm 0.008$. Figure 3 is a scatter diagram for this last-mentioned relationship and shows also the regression line.

As previously stated, committee grade is not an exact measure of firmness since some error due to the human factor is involved. More-

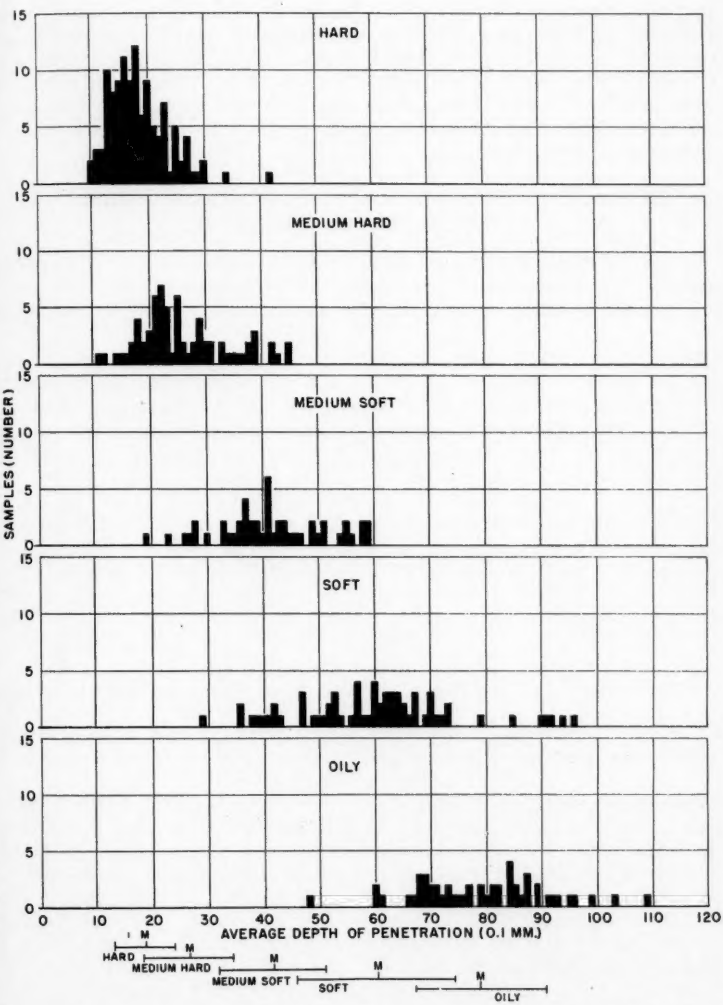

FIGURE 2.—Frequency distributions of firmness determinations made on hard, medium-hard, medium-soft, soft, and oily samples of fatty tissue, on the basis of depth of penetration by penetrometer. For each distribution the mean (M) and standard deviation of the penetrometer determinations are shown.

over, it is possible that the standards of firmness of the graders are subconsciously changed from time to time. In support of this point there are indications that in the work at Beltsville, during a period of 20 years, there has been a slight tendency to lower this standard of firmness. This is shown by comparison of the average refractive-index values in table 1 of this paper with those of Hankins and Ellis,[7] table 1. If there were no error in the committee grades and if the same principle were involved in the manual and penetrometer tests, the correlation between committee grade and penetrometer determination would approach 1 very closely.

ESTABLISHING TENTATIVE GRADE LIMITS IN TERMS OF PENETROMETER READINGS

With overlapping of grades, as indicated by penetrometer determinations or any other constant, and a lack of homogeneity of the variances[8] in the five groups, the investigator is confronted with a somewhat

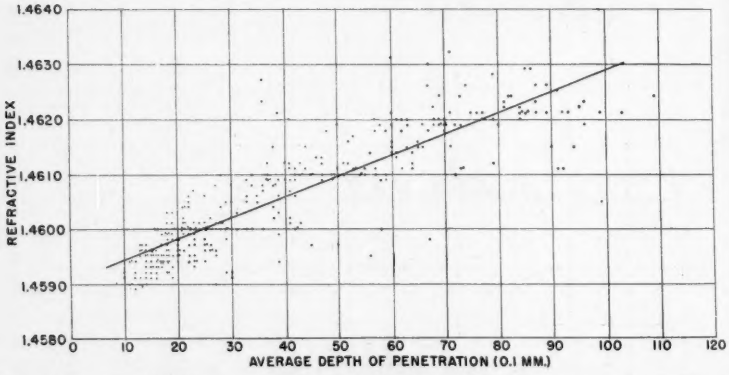

FIGURE 3.—Scatter diagram and regression line of refractive index and penetrometer determinations as indication of firmness of 351 samples of back fat.

perplexing problem in attempting to designate firmness grade limits. After considering several possible procedures in the study reported here, the writers reached the conclusion that the averages of penetrometer determinations for each of the five grades and the respective standard deviations should be used as a basis in establishing the tentative limits for these grades. After accepting 18.6 and 26.1 as means and 5.3 and 8.1 as standard deviations (table 1) for hard and medium-hard samples, respectively, the problem was to designate the most satisfactory maximum determination for the former grade and the minimum determination for the latter. It seems obvious from the foregoing that samples with penetrometer determinations 19 and 20 would be classified as hard, whereas those with determinations 25 and 24 would be classified as medium hard. Samples with intermediate determinations 21, 22, and 23 are as yet unclassified. Table 2 shows

[7] HANKINS, O. G., and ELLIS, N. R. See footnote 5.

[8] For the method employed see pp. 206–207 of SNEDECOR, GEORGE W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. 1940.

the deviations of each of these three determinations from the two means and the proportion of each deviation in terms of the corresponding standard deviation.

TABLE 2.—*Deviations of penetrometer determinations [1] of 21, 22, and 23 from the means for hard and medium-hard grades and the proportion of each deviation in terms of the corresponding standard deviation*

Penetrometer determination [2] (0.1 mm.)	Deviation from mean for hard grade	Proportion of deviation in terms of standard deviation for hard grade	Deviation from mean for medium-hard grade	Proportion of deviation in terms of standard deviation for medium-hard grade
		Percent		*Percent*
21	2.4	45	5.1	63
22	3.4	64	4.1	51
23	4.4	83	3.1	38

[1] See footnote 1 of table 1.
[2] Represents values for average of outer and inner layers of back fat.

As shown by the table, in the case of the penetrometer determination 21 its deviation from the mean for hard samples is only 45 percent of the standard deviation of such samples. On the other hand, its deviation from the mean for medium-hard samples is 64 percent of the standard deviation for samples of that grade. In view of the greater probability of the value 21 falling within the standard deviation for the hard grade, one would be justified in assigning it to that grade rather than to medium hard. Further comparison of the percentages in the third and fifth columns of table 2 indicates that the determinations 22 and 23 should be included in the latter grade. Therefore, the penetrometer determination 21 is offered as the tentative upper limit of the hard grade, whereas 22 is offered as the tentative lower limit of the medium-hard grade.

With the same procedure, the upper limit for medium-hard grade and limits for the other grades were determined. The proposed limits for the five grades are as follows: Hard, 21 or less; medium hard, 22 to 33, inclusive; medium soft, 34 to 48; soft, 49 to 70; oily, 71 or more

SUMMARY AND CONCLUSIONS

Study was made of 351 back-fat samples from hog carcasses that had been chilled for 72 hours at 33° to 35° F. (0.56° to 1.67° C.) to determine the usefulness of a penetrometer for measuring their firmness. Another object was to establish tentative grade limits in terms of the penetrometer determinations for each of five degrees of firmness—hard, medium hard, medium soft, soft, and oily. A penetrometer of standard type designed to determine firmness or consistency of foods and other materials was employed. The penetrometer needle was 3.0 inches long and 0.15 inch in diameter. The radius of the rounded, working point of the needle was 0.075 inch. A weight of 255.65 gm. and a time interval of 15 seconds were used. Penetration was determined in tenths of a millimeter. The study was conducted in 1938 and 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

The average penetrometer determinations for the back-fat samples, divided into the five degrees of firmness by a committee of three

qualified judges, increased from 18.6 for hard to 78.9 for oily samples. This represents a difference of 60.3 units of measurement, or 6.03 mm. Average refractive-index readings for these samples also showed an increase—from 1.4595 for hard to 1.4622 for oily samples.

Correlation coefficients showed that a close relationship existed between (1) the committee grade for firmness and penetrometer determination, (2) committee grade for firmness and refractive index, and (3) penetrometer determination and refractive index.

For the several grades of firmness the following limits in terms of penetrometer determinations have been established tentatively: Hard, 0 to 21, inclusive; medium hard, 22 to 33; medium soft, 34 to 48; soft, 49 to 70; and oily, 71 or more.

It is obvious that the depth of penetration for any given degree or grade of firmness depends at least on the shape and size of the needle point, the weight of the working unit, and the period during which the needle is allowed to penetrate into the sample. By changing one or more of these three factors possibly more useful grade means and limits could be established. That is a problem for future study.

THE HEMICELLULOSES OF SUGARCANE FIBER (BAGASSE)[1]

By B. L. DAVIS, *junior chemist*, and MAX PHILLIPS, *senior chemist, Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture*

INTRODUCTION

Although that fraction of the carbohydrates of plants which Schulze (*13*)[2] designated as "hemicelluloses" has been investigated by chemists for about 50 years, the chemistry of this class of substances is still in a rather confused state. A brief review of the chemical literature on hemicelluloses has been given in two previous papers (*10*, *15*) published by this Bureau. For a more extensive critical résumé of the literature, the reader is referred to a book by Norman (*8*).

There is considerable information in the literature on the chemical composition (as determined by conventional methods) of sugarcane (*Saccharum officinarum*) fiber (bagasse). The composition of the hemicelluloses of this substance, however, has been the subject of only two chemical examinations. Prinsen Geerligs (*12*) isolated the hemicelluloses of bagasse in an impure state by extraction with cold 5-percent aqueous sodium-hydroxide solution and precipitation with acetic acid and alcohol. Upon hydrolysis with boiling 2-percent sulfuric acid, the dark gummy mass yielded a sugar that had a specific rotation of +20° and was undoubtedly xylose. In addition to this sugar, there were indications that in the hydrolysate there was probably present "another sugar with a higher rotatory power, perhaps arabinose or dextrose proceeding from the hydrolysation of cellulose."

Browne (*2*) digested sugarcane bagasse (which had been freed of sugar) for 3 hours in a boiling water bath with 5-percent aqueous sodium-hydroxide solution, concentrated the aklaline extract to one-third of its original volume, and precipitated the crude hemicelluloses with ethanol. This crude material was thoroughly ground up in a mortar with an excess of alcoholic hydrochloric acid, then was washed with 80-percent ethanol until free of acid, and finally was washed with 95-percent ethanol and ether. On hydrolysis with 4-percent hydrochloric acid, the product afforded *d*-xylose, together with a small quantity of *l*-arabinose.

The investigation reported in this paper was undertaken for the purpose of determining the character and composition of the hemicelluloses of sugarcane fiber (bagasse) by methods recently developed.

METHODS AND RESULTS

A quantity of sugarcane (variety Co. 281) from the field headquarters of the Bureau of Agricultural Chemistry and Engineering at the United States Sugar Plant Field Station, Houma, La., was crushed in a small laboratory mill, and the bagasse was cut into small pieces,

[1] Received for publication February 26, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 246.

dried in a steam drier, and then air-dried. A portion of this was ground in a Wiley mill fine enough to pass a 60-mesh sieve and dried in the oven at 105° C. The following constituents were determined (in percent):[3] Ash, 2.37; nitrogen, 0.24; uronic acids (as anhydrides), 4.1; pentosans, 22.06; methoxyl 2.46; lignin, 9.09; methoxyl in ash-free lignin, 15.9. (All results, except the percentage of ash, were calculated on the basis of oven-dried and ash-free material. The percentage of ash was calculated on the basis of oven-dried material.)

The bulk of the air-dried bagasse was extracted for 30 hours with a 1:2 alcohol-benzene solution in a continuous extraction apparatus. The extracted material was heated on the steam bath until free of solvent and then ground in a Wiley mill fine enough to pass a 60-mesh sieve. To 800 gm. of the extracted bagasse (795.2 gm. of moisture-free material), 8 liters of hot water (85° C.) was added. The mixture, stirred mechanically, was digested for 2 hours at 85° C. It was then filtered, and the residual material was again digested with hot water under the conditions just described except that the digestion period was increased to 16 hours. The aqueous extracts were combined, filtered, and concentrated under reduced pressure to approximately 1 liter. To the concentrated extract was added 3.5 volumes of 95 percent ethanol. The precipitate was filtered off, washed with graded strengths of ethanol,[4] and dried in vacuo over anhydrous calcium chloride. The precipitate weighed 10 gm.

The bagasse that had been extracted with hot water was treated with 10 times its weight of hot (85° C.) 0.5-percent aqueous ammonium oxalate solution, and the mixture was digested for 24 hours at 85° C. During part of this digestion period the reaction mixture was stirred mechanically. It was then filtered, and the filtrate obtained in this extraction operation, like that from the previous one, was concentrated under reduced pressure. To the concentrated solution 3.5 volumes of 95-percent ethanol, which had been acidified with hydrochloric acid, was added. The precipitate was separated with the aid of the centrifuge, washed with 75-percent ethanol until free of oxalate, and finally was washed with graded strengths of ethanol and with ether. It was dried in vacuo over phosphorus pentoxide in the Abderhalden drier at 56° C. The crude pectin thus obtained weight 7.5 gm.

PARTIAL DELIGNIFICATION

The bagasse that had been extracted with 0.5-percent ammonium oxalate solution was washed with cold water and then treated with 10 times its dry weight of an alcoholic sodium hydroxide solution (made by dissolving 20 gm. of sodium hydroxide in 150 cc. of water and 800 cc. of 95-percent ethanol). The mixture was digested at room temperature for 24 hours, during which it was stirred intermittently with a mechanical stirrer. The plant material was filtered off and digested twice with alcoholic sodium hydroxide, as previously described. The combined alkaline alcoholic extract was neutralized with hydrochloric acid, and the solution was concentrated at 50° C. under

[3] Ash was determined by burning the sample in an electric muffle at 600° C. Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, and pentosans by the Tollens-Kröber procedure, as described by the Association of Official Agricultural Chemists (*1*). The uronic acids were determined according to the procedure recommended by Dickson, Otterson, and Link (*3*), as modified slightly by Phillips, Goss, and Browne (*11*). The percentage of methoxyl was determined by the method described by Phillips (*9*). The methyl iodide was absorbed in pyridine. Lignin was determined by the method of Goss and Phillips (*4*).

[4] The term "graded strengths" of ethanol as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol.

reduced pressure. The concentrated solution was acidified with hydrochloric acid, and the precipitated lignin was filtered off, washed with water until free of acid, and then dried in vacuo at 60° C. The yield of lignin amounted to 33 gm. It was a yellow amorphous material. No furfural was obtained when this lignin was distilled with 12-percent hydrochloric acid.

The filtrate from the lignin precipitate was neutralized with sodium hydroxide solution and evaporated to dryness on the steam bath. When the residue was distilled with 12-percent hydrochloric acid no furfural was obtained, indicating that in all probability the method of delignification used did not result in the removal of any of the furfural-yielding constituents.

ISOLATION OF THE CRUDE HEMICELLULOSES

The residual bagasse from the delignification operation described above was heated on the steam bath until the alcohol had evaporated. It was then mixed with sufficient 5-percent aqueous sodium hydroxide solution to make a thin suspension, and the mixture was allowed to digest at room temperature for 24 hours. The reaction mixture was stirred from time to time. At the end of the 24-hour period, the mixture was filtered, and the digestion of the residual material with 5-percent aqueous sodium hydroxide solution was repeated three times. To the combined alkaline extracts 3 volumes of 95-percent ethanol was added, and the precipitated hemicelluloses were separated with the aid of the centrifuge. The product was washed with ethanol, to which a little acetic acid had been added, until free of alkali and was then washed with ethanol alone and finally with ether. The hemicellulose preparation was dried in vacuo over anhydrous calcium chloride, which freed it from most of the ethanol and ether. It was then moistened with an equal weight of water and chlorinated for 1 hour, the procedure described in a previous publication (*15*) being generally followed. The chlorinated product was mixed with enough 95-percent ethanol to form a thin suspension, the mixture was well shaken, and the alcohol was separated with the aid of the centrifuge. To the residual material a 3-percent ethanolamine solution in 95-percent ethanol was added, and the mixture was heated for 10 minutes under a reflux condenser in a water bath maintained at 80° C. The reaction mixture was allowed to cool to 50° C. and then was centrifuged. The supernatant liquid was drawn off, and the extraction with ethanolamine solution was repeated. The extracted hemicelluloses, after being washed with 95-percent ethanol, were suspended in a 5-percent aqueous sodium hydroxide solution and precipitated by the addition of three volumes of 95-percent ethanol. The hemicelluloses were separated with the aid of the centrifuge, washed with neutral 70-percent ethanol, then with 70-percent ethanol acidified with acetic acid, and again with neutral 70-percent ethanol. The material was then washed with graded strengths of ethanol and finally with anhydrous ether. It was dried first in a vacuum desiccator over anhydrous calcium chloride at room temperature and finally in a vacuum oven at 40° C. The yield amounted to 117 gm. An analysis of this product gave the following results (in percent): Ash, 1.58; nitrogen, 0.03; uronic acids (as anhydrides), 4.96; pentosans, 95.36; lignin, 0.20; methoxyl, 0.50. (All percentages except that for ash

were calculated on the basis of moisture-free and ash-free material. The percentage of ash was calculated on moisture-free material.)

HYDROLYSIS

In a preliminary experiment the hemicellulose preparation (35.4 gm. of moisture-free and ash-free material) was treated with 2,000 cc. of 2.5 percent sulfuric acid, and the mixture was boiled under a reflux condenser for 15 hours. The reaction mixture was allowed to cool, and the dark insoluble material was filtered off. To the filtrate approximately nine-tenths of the calculated quantity of barium hydroxide solution was added slowly, while the reaction mixture was stirred mechanically and the temperature was maintained at 40° C. An excess of powdered barium carbonate was then added, and the mixture was heated at 70° C. until the acid was completely neutralized. Some decolorizing carbon (Norit) was then added, and the mixture was heated for another 15 minutes. It was then filtered, the insoluble material was washed with hot water, and the washings were added to the main filtrate. The filtrate was concentrated to a volume of approximately 150 cc. under reduced pressure at a temperature not exceeding 50° C. The concentrated solution was poured slowly, with stirring, into four volumes of absolute ethanol. The precipitated barium salt was separated with the aid of the centrifuge. After the clear supernatant liquid was drawn off, the barium salt was washed with absolute ethanol and with anhydrous ether and was then dried in vacuo over sulfuric acid. The dried material weighed 1.2 gm.

The filtrate from the barium salt was decolorized with carbon (Norit) and concentrated under reduced pressure at 50° C. to a thin sirup. This was poured, with stirring, into 4 volumes of absolute ethanol, and only a slight precipitate was produced. The alcoholic solution was centrifuged, and the clear supernatant liquor separated from the small quantity of gummy matter was again concentrated under reduced pressure at 50° C. The sirup was allowed to remain in a desiccator over anhydrous calcium chloride until crystals began to form. It was then allowed to remain in an electric refrigerator until a considerable quantity of sugar had crystallized out and the mixture was then filtered. The sugar was recrystallized from 75 percent ethanol, and after drying in the vacuum desiccator over sulfuric acid it weighed 16.0 gm. This sugar was identified as *d*-xylose by its melting point, by the refractive indices of its crystals,[5] and by Bertrand's method (*5*). Bertrand's double cadmium salt was also identified by determining the refractive indices of its crystals and comparing them with a known specimen of this salt.

From the sirup remaining after the separation of the *d*-xylose crystals, a diphenyl hydrazone was prepared. This was identified as the diphenyl hydrazone of *l*-arabinose by its melting point and by the optical properties of the crystals. On oxidation with nitric acid the sirup yielded oxalic acid but no mucic acid or saccharic acid. Both the Pinoff (*5*) test for fructose and the test for mannose were negative.

The barium salt obtained in the hydrolysis of the hemicellulose preparation described above gave Tollens' (*14*) naphthoresorcinol test for uronic acids. On oxidation with either nitric acid or bromine

[5] All identifications by optical methods were made by G. L. Keenan of the Microanalytical Division of the Food and Drug Administration, Federal Security Agency.

water, it gave no mucic acid, thus indicating the absence of galacturonic acid. The barium salt was dissolved in water, and the calculated quantity (less 5 percent) of standard sulfuric acid solution required to liberate the combined acid was added, and the mixture was warmed for 15 to 20 minutes at 60° C. The barium sulfate was then filtered off, and the filtrate was concentrated under reduced pressure to 50 cc. (the temperature of the bath during concentration did not exceed 45°). The concentrated solution was poured into 200 cc. of absolute ethanol, and the mixture was allowed to stand overnight, after which the excess barium uronate was filtered off. The alcoholic filtrate was distilled under reduced pressure at a temperature not exceeding 45°, and the concentrated solution was diluted with water to a volume of 40 cc. To this solution was added a quantity of solid cinchonine somewhat in excess of that required for complete neutralization of the acid, and the mixture was warmed on the steam bath for 15 minutes. The excess of cinchonine was filtered off, and the filtrate was extracted with two 20-cc. portions of chloroform. The aqueous solution was concentrated in the vacuum desiccator over calcium chloride. The cinchonine salt obtained was recrystallized from water. It melted at 190° C. (corrected). When mixed with some pure cinchonine glucuronate, the mixture melted at 189° (corrected). It was not possible to establish the identity of the salt by optical methods, because the refractive indices of the crystals of cinchonine glucuronate and of cinchonine galacturonate were found to be so nearly alike as to be indistinguishable.

The brucine salt was prepared in a similar manner. When the melting point of the salt was determined, the following was noted: At about 170° C. the material shrank away from the side of the melting-point tube, and at 193°–195° (corrected) it melted without any noticeable decomposition. The same behavior was noted when the melting point of a known and pure specimen of brucine glucuronate was determined. As in the case of the cinchonine salt, it was impossible to establish the identity of the salt by optical methods and for the same reason.

It cannot be said that the identity of the uronic acid was established beyond any doubt; nevertheless, the melting points of the two salts, together with the fact that no mucic acid was obtained on oxidation, are strong indications that the uronic acid obtained was glucuronic acid.

A second batch of hemicellulose, corresponding to 44.33 gm. of moisture-free and ash-free material, was hydrolyzed with 2,500 cc. of 2.5-percent sulfuric acid, following the procedure described above. After removal of the barium uronate, the alcohol was removed from the solution under reduced pressure at 50° C., after which the concentrated sugar solution was diluted with water to a volume of 500 cc. The total reducing sugars in this solution, as determined by the method of Munsen and Walker (*1*) amounted to 39.94 gm. (calculated as glucose). When a 3-cc. aliquot of the sugar solution was distilled with 12-percent hydrochloric acid, and the distillate was treated with phloroglucinol solution, following the procedure used for the determination of pentosans, 0.2366 gm. of phloroglucide precipitate was obtained. This is the equivalent of 20.92 gm. of furfural in the total solution. The sugar solution was also analyzed for arabinose by the Wise and Peterson (*16*) modification of the method of Neuberg and Wohl-

gemuth (*7*), and was found to contain a total of 1.40 gm. of arabinose. The percentage of furfural afforded by the hemicellulose preparation, when distilled with 12-percent hydrochloric acid, was also determined.

The analytical data and the composition of the hemicellulose preparation calculated from these data are as follows (in percent):[6] Uronic acid (as anhydride), 4.96; total furfural, 55.99; furfural from uronic acid, 0.94; *l*-arabinose, 3.70; furfural from *l*-arabinose, 1.77; furfural from *d*-xylose (by difference), 53.28; *d*-xylose (calculated from furfural), 92.5; molar ratio of uronic acid to *l*-arabinose and *d*-xylose, 1:0.87:21.9.

SUMMARY

The hemicelluloses were isolated from sugarcane fiber (bagasse) which had previously been freed of sugars and pectin. The product obtained was light gray and was practically free of nitrogenous substances and lignin. On hydrolysis with dilute sulfuric acid it afforded *d*-glucuronic acid, *l*-arabinose, and *d*-xylose in the approximate molar ratio of 1:0.87:21.9.

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FACTORS INFLUENCING RATE OF GAIN OF BEEF CALVES DURING THE SUCKLING PERIOD [1]

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INTRODUCTION

The following study was undertaken as part of a broad project to evaluate the factors that influence the efficiency of feed utilization in cattle. One of the objects of a record-of-performance program with cattle is to devise a means of evaluating growth ability and efficiency of gain at an early age. Knapp, Lambert, and Black (*5*) [2] have shown that birth weight is of limited value as an index of a calf's growth potentialities. They showed that a calf's prenatal growth is primarily an expression of the dam, and therefore that little of the variation in prenatal growth can be attributed to genetic differences between calves.

The next period of development, the suckling period, is at first one of adjustment to a new environment following parturition. Milk makes up the larger portion of the diet, though in the latter part of the suckling period other feeds gradually replace it. Since milk is an excellent food for growth, the suckling period is one of large skeletal increase. Approximately 80 percent of the mature skeletal size of cattle has been attained by the end of the suckling period, whereas only about 40 percent of the mature weight has been reached. If it were possible to evaluate an animal by the end of the suckling period, a great amount of time and labor might be saved. The study reported in this paper was made to determine the factors that influence the rate of gain in calves during the suckling period and also to ascertain the value of rate of gain during this period as a basis of selection for breeding.

PREVIOUS INVESTIGATION

Donald (*3*), as a result of a study of milk consumption and growth of suckling pigs, stated:

> The effect of an investigation such as this is to invite reconsideration of the importance of variability in weaning or 3-week weight. Differences in size among young pigs are probably useless for comparing their genetic quality. Such differences as have been found, and even some deaths, can be satisfactorily explained by variations in the level of nutrition.

He also stated:

> The lightest pigs at or before weaning are by no means always the "poorest doers" in later life.

Donald (*4*) also made a comparison of pigs reared by foster mothers with those reared by their own dams. Half of each litter of pigs

[1] Received for publication February 24, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 254.

was transferred to a foster mother and half remained with the natural dam. Thus half of the pigs suckled by each sow were foster pigs and half were her own. It was found that the milk production of the sow and other influences attributable to the sow had a greater effect on the gains of the animals than did any other sources of variation.

Comstock [3] found that there was no correlation between weaning weight and postweaning growth in swine when growth rate was calculated at a comparable stage. He suggested that the absence of correlation may be due partly to growth limitations of some of the pigs during the suckling period and partly to different genes controlling growth during the suckling and postsuckling periods. These results are in entire agreement with the observation of Donald (*3*).

Black and Knapp (*1*), in a study of record-of-performance steers, showed that there was no correlation between efficiency of gain before and after weaning. They also noted a low negative correlation in rate of gain before and after weaning. The lack of correlation in both efficiency and rate of gain may have been due to inhibition in growth, during the suckling period, of steers with inherently high efficiency because of limited nutrients, their true inherited efficiency being displayed after weaning when feed was not limited.

MATERIAL AND METHODS

For the present study the records of 58 beef Shorthorn calves from the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., and 180 Hereford calves from the United States Range Livestock Experiment Station, Miles City, Mont., were used. The Shorthorn calves were sired by 8 bulls and the Hereford by 3. The study was made at Beltsville in 1940 from data obtained in 1931–36 for other purposes. The data available from Beltsville included a record of sires, dams, sex, birth weights, weaning weights, and the quantities of milk, grain, and hay consumed by each calf. Milk consumption was calculated by weighing the calf 1 day each week before and after nursing. Both grain and hay were fed individually, and each calf was allowed all it would consume. The grain mixture consisted of 4 parts by weight of cracked corn, 3 parts of crushed oats, 2 parts of wheat bran, and 1 part of linseed meal. The hay fed was No. 2 leafy alfalfa.

In the study involving rate of gain as a basis of selection for breeding, bull and steer calves at Beltsville were used, selection of the bull calf being made at 140 days of age. At that time all bull calves were judged for beef characteristics. Bulls that failed to meet the standard set up were castrated and fed out as steers. Castration was bloodless and had little or no effect on the animals at the time. The dams of the calves also were scored for beef characteristics at irregular intervals over a period of years.

The data available from Miles City included a record of sires, dams, sex, birth weights, weaning weights, and ages at weaning. The calves were weaned at an average age of 180 days and were not grain-fed up to weaning.

[3] COMSTOCK, R. E. THE RELATIONSHIP BETWEEN WEANING WEIGHT AND POSTWEANING RATE. [Unpublished.]

A correlation study of the Beltsville data was made to determine the effect of consumption of different quantities of milk, grain, and hay on rate of gain during the suckling period. Both the Beltsville and Miles City data were analyzed to determine the variance of daily gain due to sires and sex of calves. Correlations and analyses of variance as outlined by Snedecor (*7*) were used in the analyses of the data.

EXPERIMENTAL RESULTS

In the study of the Beltsville data, the following correlations were found:

	Correlation coefficient [1]
Simple correlation between—	
Daily gain and quantity of milk	**0. 517
Daily gain and quantity of grain	. 209
Daily gain and quantity of hay	. 121
Quantities of milk and grain	. 142
Quantities of milk and hay	**. 388
Quantities of grain and hay	**. 456
Multiple correlations between daily gain and quantities of—	
Milk and grain	**. 591
Milk and hay	**. 624
Grain and hay	. 211
Milk, grain, and hay	**. 641
Partial correlations between daily gain and quantity of—	
Milk (grain and hay constant)	**. 619
Grain (milk and hay constant	. 188
Hay (milk and grain constant)	*. 308

[1] * Statistically significant, $P < 0.05$. ** Highly significant, $P < 0.01$.

The simple correlations indicate that of the feeds consumed the quantity of milk had the greatest effect on rate of gain, followed in order by grain and hay. These last two correlations are not statistically significant, however. There was a slight tendency for the calves that consumed the most milk to consume the least hay and grain. The correlation between the quantities of grain and hay consumed is significant. The multiple correlations indicate that gain per day is associated with these three variables, which account for as much as 41 percent of the variation in rate of gain. Partial correlations show that the quantities of milk and of hay consumed are significantly correlated with daily gain but that the quantity of grain consumed is not significantly correlated with rate of gain. This finding seems to indicate that the more bulky feeds, milk and roughage, have the greatest effect on rate of gain, whereas grain, a more concentrated feed, has little effect. This is in agreement with observations made by animal husbandmen.

TABLE 1.—*Anolysis of variance of daily gains, during the suckling period, of 58 Shorthorn calves sired by 8 bulls at Beltsville, Md.*

Variation	Degrees of freedom	Sum of squares	Mean square [1]
Due to sex [2]	2	1. 3582	**0.6791
Due to sire effects	14	1. 1857	.0847
Between calves of the same sex and from the same sire	41	1. 8305	.0446
Total	57	4. 3744	.0767

[1] ** $P < 0.01$, highly significant.
[2] Animals were sorted on the basis of 3 sex differences; there were 14 bulls, 15 steers, and 29 heifers.

Table 1 shows the variance of daily gains, due to sex and sires, of the calves from Beltsville. The analysis shows that differences between sexes are statistically significant, whereas differences between the progeny of different sires are not significant. By analysis of covariance, differences due to sex in the correlations were eliminated. The correlations for calves of the same sex indicate little change in the relationship between quantity of feed and rate of gain. The multiple relationship was reduced from 0.641 to 0.529. The indications are, however, that approximately the same relationship exists between rate of gain and the quantities of milk, grain, and hay consumed among calves of the same sex as among those of the entire population. There were no significant yearly effects owing to the fact that the calves were born during all seasons of the year, causing a larger variation within year than between years.

Further analyses of the Beltsville data show that bull calves consumed significantly more milk than steer calves, but there were no significant differences in the quantities of grain and hay consumed by bulls and steers. This finding seems to indicate that the superior beef characters of the bull calves retained for breeding were due to the greater quantities of milk consumed during the suckling period. As already stated, significant differences in daily gains between the progeny of sires were not demonstrated. Since milk production of the cows bred to different sires may vary either through accidential or premeditated selection of the cows for high milk production, the use of daily gain during the suckling period as a measure of genetic superiority of the progeny for growth is subject to question.

In determining the value of rate of gain as a basis for selection, the analysis of variance of gains, milk consumption, and scores of dams and calves gave the following results: Calves saved for bulls made the most gain up to 140 days of age; they were from cows that gave the most milk up to 140 days; and they were from the poorest scoring cows for beef characteristics. The first two results indicate that selections were associated with nutritional differences and that as a result of such selection the milk production of the herd would be maintained at a high level. This high milk-production level would result in a comparatively low cost per pound of feeder calf. However, the fact that the bull calves selected were from the poorest scoring cows for beef characteristics indicates that the method of selection used would result in poorer beef qualities in the herd. These qualities may not show in the animals selected but would be likely to do so in their progeny. For example, at Beltsville, one of the herd bulls sired thick-fleshed steer calves as shown by the physical analysis of their carcasses. A son of this herd sire, selected as shown above, was out of a cow of poor beef quality but of high milk production. His progeny had significantly more bone and less fat than their sire.

A similar condition of selection was reported by Phillips and Dawson (*6*) in sheep. They found that in the selection of lambs for breeding single lambs were favored over twins, early lambs over late lambs, and heavy lambs at birth over light lambs, although no conscious effort was made to favor any of these groups.

As already stated, an analysis-of-variance study was made of the daily gains of 180 calves sired by 3 bulls at Miles City, Mont. Black and Knapp (*2*) observed significant differences, in rate of gain subse-

quent to weaning, in the progeny of the three bulls. Table 2 shows the analysis of variance of the daily gains prior to weaning. With these calves, as well as those from Beltsville, significant differences were observed between sexes but not between the progeny of different sires. The small differences that were observed between the progeny of different sires are probably the result of the nutritional level rather than of genetic variation.

TABLE 2.—*Analysis of variance of daily gains, during the suckling period, of 180 Hereford calves sired by 3 bulls at Miles City, Mont.*

Variation	Degrees of freedom	Sum of squares	Mean square [1]
Due to sex	1	0.4080	**0.4080
Due to sire effects	4	.2788	.0697
Between calves of the same sex and from the same sire	174	6.6785	.0384
Total	179	7.3653	.0411

[1] $^{**}P<0.01$, high'y significant.

Since the study showed no significant difference in rate of gain during the suckling period in the progeny of different sires, it may be concluded that rate of gain during this period is no indication of the genetic superiority of the calves. However, determination of rate of gains during the suckling period would be of value if this gain were shown to have a close correlation with gain after weaning. A correlation study on record-of-performance Hereford steers at Miles City showed a coefficient of 0.023 (nonsignificant) between rate of gain before and after weaning. This observation is in agreement with that of Black and Knapp (*1*) with cattle, and with those of Donald (*3*) and Comstock [4] with swine. The conclusion seems sound, therefore, that there is little or no relationship between rate of gain during the suckling period and rate of gain after weaning. Consequently, as a method of genotypic selection, observations on rate of gain prior to weaning have little value.

SUMMARY

The study reported in this paper was made to determine the factors influencing rate of gain in beef calves during the suckling period and to ascertain the value of rate of gain during this period as a basis of selection for breeding.

A statistical study of the daily gains during the suckling period was made on 58 beef Shorthorn calves at the United States Department of Agriculture Beltsville Research Center, Beltsville, Md., and 180 Hereford calves at the Range Livestock Experiment Station, Miles City, Mont. The work was carried on at Beltsville in 1940 with data obtained in 1931–36 for other purposes. The influence, on the daily gains, of sires, dams, sex birth and weaning weights, ages at weaning, and milk, grain, and hay consumption, was determined.

Partial correlation studies indicated that under the conditions of this study, milk consumption had the greatest influence on rate of gain, followed in order by hay and grain. The combined influence of these three variables accounted for 41 percent of the variation in rate of gain during the suckling period.

[4] See footnote 3.

The analysis-of-variance study indicated that differences between the progeny of different sires cannot be demonstrated by rate of gain during the period prior to weaning. Furthermore, evidence is presented which shows that there is little or no relationship in rate of gain before and after weaning. Sex was found to have a significant influence on rate of gain. The study indicated that when selection of breeding animals was made during the suckling period, the calves selected were those that made the greatest gain and that they were from cows which gave the most milk but scored the poorest for beef characteristics.

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